Eco-hydrogeomorphic Interactions in Streams of Ulu Temburong National Park, Borneo

Baker, Kate

Awarding institution:
King's College London

ECO-HYDROGEOMORPHIC INTERACTIONS IN STREAMS OF ULU TEMBURONG NATIONAL PARK, BORNEO

Kate Baker

0614898

A thesis submitted to King's College London
for the degree of Doctor of Philosophy

DEPARTMENT OF GEOGRAPHY
KING'S COLLEGE LONDON

Abstract

This PhD thesis focuses on the basic eco-hydromorphic dynamics of pristine tropical stream systems on the island of Borneo. Exploring theories and models developed in temperate streams and rivers to determine if they can be applied or appropriately adjusted to describe and explain some of the tropical stream processes. Streams in Ulu Temburong National Park in Brunei Darussalam are used as a case study owing to its pristine rainforests and intact stream catchments that have hitherto been little studied. The first part of the thesis assesses the distribution of macroinvertebrates in flow biotopes and investigates if the arrangement, structure and juxtaposition of the flow biotopes influences macroinvertebrate assemblages and population. It is demonstrated that the more consistent environmental conditions of waterfalls and cascades, in comparison to the mixed-substrate biotopes (pools and riffles), have a strong influence on the macroinvertebrate communities. The influence of discontinuity on stream biota and ecosystem functions was explored at confluences zones and in pools above-and-below waterfalls. Results suggested there was a stronger link between confluence hydrology and macroinvertebrates, than with organic matter and periphyton. The waterfall study found varied effects of biotic and abiotic factors on community structure and ecosystem function. Higher fish densities were in below-waterfall pools and higher shrimp abundance in above-waterfall pools. However, macroinvertebrate densities (excluding shrimp) were similar among both pool types. Ambient periphyton was higher in below-waterfall pools but leaf litter decomposition rates did not differ, suggesting that neither shrimp nor fish densities had consistent impacts on this ecosystem function. The trophic structure of the macroinvertebrates living on waterfalls was investigated using stable isotope analysis (SIA; $\delta^{13}C$ and $\delta^{15}N$ of leaf litter and periphyton) and gut contents analysis (GCA), establishing potentially three trophic levels. This thesis successfully used models developed in temperate streams to develop an understanding of the basic dynamics of pristine tropical stream systems in terms of eco-hydromorphology, which will assist with sensible conservation management and for robust ecosystem monitoring.

Insert Photo: Author conducting fieldwork on Sungai Lower Apan (photo taken by Hanyrol Ahmad Sah)

Table of Contents

TABLE OF CONTENTS 4

LIST OF FIGURES 9

LIST OF TABLES 15

ACKNOWLEDGEMENTS 17

CHAPTER 1: INTRODUCTION & RESEARCH AIMS 19

1.1 Motivation and Context 19

1.2 Research Aims and Objectives 22

1.2.1 Overview 22

1.2.2 General Aims and Objectives 22

1.2.3 Research Questions and Hypotheses 22

1.3 Research Methodology 24

1.4 Research Novelty 24

1.5 Research Challenges 25

1.6 Thesis Outline 26

CHAPTER 2: LITERATURE REVIEW 29

General Introduction 29

2.1 Introduction to macroinvertebrates 29

2.2 Introduction to eco-hydromorphology 30

2.3 Stream classification model research 31

2.3.1 Introduction 31

2.3.2 Key statistical models 33

2.3.3 Key Conceptual based models 39

2.4 Tropical Stream research 45

2.5 Summary 47

CHAPTER 3: BACKGROUND TO RESEARCH SITES IN ULU TEMBURONG NATIONAL PARK 49

3.1 General Introduction 49

3.2 Introduction to Brunei 49

3.3 Climate in Brunei and Ulu Temburong National Park 50

3.4 Landform processes in Ulu Temburong National Park 53

3.5 Rivers and streams of Ulu Temburong National Park 54

3.5.1 Geomorphology and physical factors 54
3.5.2 Background community structure 58
3.6 Summary 60

CHAPTER 4: ECO-HYDROMORPHIC CLASSIFICATION FOR UNDERSTANDING TROPICAL STREAM MACROINVERTEBRATE BIODIVERSITY 62
4.1 Introduction 62
4.2 Methods 63
4.2.1 Study Sites 63
4.2.2 Field Methods 64
4.2.3 Laboratory Methods 65
4.2.3 Data Analysis 66
4.3 Results 67
4.3.1 Site Description 67
4.3.2 Biotope Taxa Distribution 69
4.3.3 Macroinvertebrate Size Structure in the Biotopes 72
4.4 Discussion 73
4.5 Conclusions 77

CHAPTER 5: MACROINVERTEBRATE DIVERSITY IN POOLS OF SUNGAI TEMBURONG & SUNGAI BELALONG: A SHORT COMMUNICATION 79
5.1 Introduction 79
5.2 Methods 81
5.2.1 Study sites and fieldwork 81
5.2.2 Sampling and Identification of Benthic Macroinvertebrates 82
5.2.3 Data Analysis 83
5.3 Results 83
5.3.1 Representative sampling 83
5.3.2 Temburong and Belalong 84
5.4 Discussion 87
5.5 Conclusion 89

CHAPTER 6: THE EFFECT OF RIVER-TRIBUTARY INTERACTIONS ON BENTHIC HABITAT & MACROINVERTEBRATE BIODIVERSITY 91
6.1 Introduction 91
6.2 Methods 95
6.2.1 Study Site 95
6.2.2 Study design 95

6.2.3 Field Methods 96
6.3 Results 99
6.3.1 Physical measurements of confluence zones 99
6.3.2 Organic matter at the confluence zones 100
6.3.3 Periphyton at the confluence zones 101
6.3.4 Biodiversity among the confluence zones 102
6.3.4 Biodiversity at river margin, river-stream interface and upstream pool 102
6.4 Discussion 106
6.5 Conclusion 109

CHAPTER 7: FLUVIAL BIOTOPES INFLUENCE MACROINVERTEBRATE BIODIVERSITY IN SOUTHEAST ASIAN TROPICAL STREAMS 111
7.1 Introduction 111
7.2 Methods 113
7.2.1 Study Sites 113
7.2.2 Field Methods 114
7.2.3 Laboratory Methods 115
7.2.4 Data Analysis 115
7.3 Results 116
7.3.1 Site Description 116
7.3.2 Biodiversity of the Study Reaches 118
7.3.3 Biodiversity among Biotopes 121
7.4 Discussion 124
7.4.1 Biodiversity of the Study Reaches 125
7.4.2 Biotope Biodiversity 126
7.4.3 Difficulties of Sampling Tropical Streams 127
7.5 Conclusion 128

CHAPTER 8: BENTHIC COMMUNITY STRUCTURE & ECOSYSTEM FUNCTIONS IN ABOVE- AND BELOW-WATERFALL POOLS IN BORNEO 130
8.1 Introduction 130
8.2 Methods 132
8.2.1 Study Sites 132
8.2.2 Background Community Structure 134
8.2.3 Physical Habitat Measurements 135
8.2.4 Macroinvertebrate, Shrimp and Fish Sampling 135
8.2.5 Ecosystem Function Estimates 137

8.3 Results 139
8.3.1 Physical Habitat 139
8.3.2 Macroinvertebrate, Shrimp and Fish 139
8.3.3 Ecosystem Function Estimates 141
8.4 Discussion 146
8.4.1 Biodiversity in above- and below-waterfall pools 147
8.4.2 Ecosystem Function 149
8.5 Conclusion 151

CHAPTER 9: MACROINVERTEBRATE TROPHIC STRUCTURE ON WATERFALLS IN BORNEO 152
9.1 Introduction 152
9.2 Methods 154
9.2.1 Study Sites 154
9.2.2 Geomorphic Measurements and Functional Habitats 155
9.2.3 Macroinvertebrate trophic structure 156
9.3 Results 157
9.3.1 Geomorphic Measurements and Functional Habitats 157
9.3.2 Macroinvertebrate trophic structure 158
9.4 Discussion 163
9.5 Conclusions 167

CHAPTER 10: SUMMARY 168
10.1 Overview of Research Aims and Questions 168
10.2 Recommendations for Future Work 175
10.3 Concluding Remarks 176
REFERENCES 178

APPENDICES 210
Appendix for Chapter 4: Eco-Hydromorphic Classification for Understanding Tropical Stream Macroinvertebrate Biodiversity 210
Appendix for Chapter 5: Macroinvertebrate Diversity in Pools of Sungai Temburong & Sungai Belalong: A Short Communication 216
Appendix for Chapter 6: The effect of river-tributary interactions on benthic habitat & macroinvertebrate biodiversity 221
Appendices for Chapter 7: Fluvial biotopes influence macroinvertebrate biodiversity in Southeast Asian tropical streams 236
Appendices for Chapter 8: Benthic Community Structure & Ecosystem Functions in Above-and Below-Waterfall Pools in Borneo 247

Appendix for Chapter 9: Macroinvertebrate trophic structure on Waterfalls in Borneo
.....260

List of Figures

Figure 1.1. Forest cover and condition in northern Borneo including Brunei, circled in black, and Malaysia as inferred from Landsat images in 2009. Indonesia is in brown. This image is adapted from Bryan (2013), with insert map showing the location of Brunei in Borneo Island.....21
Figure 1.2 Thesis overview and the main conclusions from each data chapters.....28
Figure 2.1. Timeline of the key theories and contributions to stream eco-hydromorphology, with a focus on aquatic classification. These concepts are not fixed in time, but usually develop, overlap and intermingle with other ideas over time. The outline of each box indicates the field in which the developments occurred: stream geomorphology (thin, dashed black line), ecology (dashed green line), stream ecology (thick green line), and integrated terrestrial-aquatic systems (thick, dashed black line). The shaded green boxes indicate aquatic classifications that this thesis focuses on. (Adapted from Melles et al., 2012).....33
Figure 2.2. View of streams in a hierarchical context. In this stream system and associated habitat subsystem, the microhabitats are part of the microscale, while pools and riffles along with reaches are part of the meso-scale and the drainage basin constitutes the macroscale (Adapted from Frissel et al., 1986).....38
Figure 2.3. Habitat template model showing the relationship of assemblage traits or species to the frequency of disturbance and complexity of the physical habitat. The shaded areas represent the different predicted traits and dashed line shows the transition point. Diagram from Ross (2013), which was adapted from Resh et al. (1994) and Townsend and Hildrew (1994).....40
Figure 2.4. Original diagram from Vannote et al (1980) illustrating the river continuum concept. The river channel is divided into three main sections. 41
Figure 2.5. Hierarchical patch dynamics view (HPD), which conceptualizes the fluvial system as nested and discontinuous patch mosaics (on the left), and the four dimensions of a river modified from Ward (1989; on the right).....45
Figure 2.6. Key tropical studies with a focus on benthic macroinvertebrates; studies have been dominated by those conducted in a few countries and regions. Countries are highlighted in various colours. 46
Figure 3.1. Borneo Island is divided into three countries, Indonesia, Malaysia and Brunei (on the left). Brunei Darussalam is shown; note that the country is split into two parts with Sarawak (Malaysia) separating the Temburong district from the rest of the country (on the right). The capital, Bandar Seri Begawan, is situated in the larger section of Brunei. Bangar is the largest town in the Temburong district. 50
Figure 3.2. Average rainfall and seasonal wind directions caused by the movement of the intertropical convergence zone. This image is from the Curriculum Development Department, Ministry of Education, Brunei Darussalam. The author has added the location of the two fieldwork sites, Kuala Belalong Field Study Centre (KBFSC) and the base camp for the Bukit Pagon expedition. 51
Figure 3.3. Average temperature (top graph) and total precipitation (bottom graph) recorded at the weather centre at Kuala Belalong Field Study Centre. Bars in white indicate when fieldwork was conducted.....52
Figure 3.4. (A) Schematic illustrating shallow and deep-seated landslides. (Image from Thinktheearth.net) (B) Shallow landslide that occurred along Sungai Esu in 2014. 54

Figure 3.5. Waterfalls in the tributaries of Sungai Temburong (top photos). Large debris dam (bottom left) on Sungai Esu, and a single tree trunk changing the flows (bottom right) from Sungai Engkabang. 55
Figure 3.6. Schematic illustrating how a tropical river channel changes from low to high flows, often daily. Low flow channel width is shown as thick dark black lines with the high flow channel width shown as dashed black lines. Blue arrows represent the water flow direction. Water can rise and fall within hours. Low flows support a mix of habitats and biotopes; high flows create a homogeneous channel and backs water up into the tributaries. Frequent landslides are a source of sediment and nutrients; waterfalls are a longitudinal block for animals and cause hydrologic disconnection from the main channel. 56
Figure 3.7. Evidence of the frequent powerful flows on Sungai Temburong and Sungai Belalong (A) A large debris dam on Sungai Temburong in April 2013, with a (circled) man for scale. (B) Photo taken in the same place but one year later, after the debris dam had been washed away. (C) Water flowing up Sungai Belalong after a storm event on Sungai Temburong. The photo was taken at Kuala Belalong Field Study Centre (KBFSC), which is approximately 300 m from the confluence. Video shown here: https://goo.gl/P1Z1i7. Photo credits: (A) and (B) were taken by the author, and (C) was taken by Teddy Chua (KBFSC manager). 57
Figure 3.8. Gastromyzon in in a pool in Sungai Baki (top left) and grazing scars in contrast to the fine layer of sediment that covers most of the rock (top right). Shrimp in Sungai Belalong (bottom left) and a flattened Mayfly Heptageniidae (bottom right). Images taken by the author. 59
Figure 3.9. Brunei, with the Temburong district shown on its eastern side (top map). The southern part of the Temburong district is Ulu Temburong National Park, Kuala Belalong Field Study Centre (KBFSC) and Pagon base camp are highlighted. All the study sites corresponding to Chapters 4–9 are highlighted in the bottom map. 61
Figure 4.1. Map and schematic of study sites A) Map of Borneo Island in S.E Asia with Brunei highlighted. B) Plan view schematic of the two study sites, tributary of 61a (left) and stream 61a (right). 64
Figure 4.2. Dendrogram of A) biotope physical conditions and B) biotope taxa abundance. The first letters represents the study site: S= Stream 61a, T= tributary. The second letter and the number represents the biotope sampling unit: R= run, RIF= riffle, P=pool. Letters in bold represent the fast flowing biotopes. 68
Figure 4.3. Bar plots with richness, density, and biomass of macroinvertebrates at the two study sites. Includes bar plots of stream 61a and tributary, the fast and slow flowing habitats, and the three biotopes (runs, riffles and pools). Error bars represent standard deviation (Stream 61a n= 6, tributary n=6, both fast and slow flowing habitats n=6, runs n=3, riffle n=3 and pool n=6). Significant differences between the fast and slow sites with richness (p=0.05*). 70
Figure 4.4. MDS ordination with a BIO-ENV that shows the environmental variables that best correlate with macroinvertebrate community structure. The biotope names have been abbreviated with the first letter representing the study site: S= Stream 61a, T= tributary. The second letters represent the biotopes: R= run, RIF= riffle, P=pool. The environmental data in grey with G=gravel, CO= cobbles, LL= leaf litter, B= boulder, BR= bedrock and WD= wood debris, M=moss. Stress: 0.01. 71
Figure 4.5. Length- frequency distributions of macroinvertebrates among stream 61a and its tributary, fast and slow biotopes, and among the three biotopes (runs, riffles and pools). Error bars represent standard deviation (Stream 61a n= 6, tributary n=6, both fast and slow flowing habitats n=6, runs n=3, riffle n=3 and pool n=6). Significant differences

found between the fast and slow sites (p= 0.04*), pool and runs (p=0.01) and riffles and runs (p=0.01) using Kolmogorov-Smirnov test. 73
Figure 4.6. Sample-size-based rarefaction and extrapolation for fast (runs and riffles) and slow (pools) flow biotopes. Graph (A) includes all sampled taxa and (B) includes the common taxa which is defined as any taxa that is found more than once. 77
Figure 5.1 Sungai Temburong during low flows 80
Figure 5.2 Photo of Sungai Belalong outside KBFSC. Photo on the left taken at 17.45 when the rain started and photo on the right taken at 20:00 81
Figure 5.3. Map of Brunei situated in the north of Borneo Island. The country is split into two parts, with Ulu Temburong National park within the Temburong district. Kuala Belalong Field Study Centre (KBFSC) is situated upstream from the Temburong- Belalong confluence. Kick samples were taken downstream from the Temburong- Belalong confluence (T1-3) and upstream from the confluence on Temburong (T4-6) and Belalong (B1-6). 82
Figure 5.4. Sample-size-based rarefaction and extrapolation for fast and slow biotopes. Graph (A) includes all sampled taxa and (B) includes the common taxa which is defined as any taxa that is found more than once. 84
Figure 5.5. Total richness, density, and biomass (ash-free dry mass; AFDM) of macroinvertebrates for Temburong and Belalong. Error bars represent standard deviations (Temburong, n = 6; Belalong, n = 6). 85
Figure 5.6. Dendrogram of benthic aquatic insect abundance at each of the sites. T=Temburong and B=Belalong. Numbers indicate replicate pools. 86
Figure 5.7. NMDS of macroinvertebrate community patterns and sampling sites. The light grey crosses represent taxon that are less than the mean, only the priority taxon (greater than the mean) are displayed. List of taxa abbreviations are in appendix Chapter 5. The dotted line provides a subjective division between the Temburong and Belalong pools. (Stress: 0.15). 87
Figure 6.1. Substrate accumulating confluence zone 8 (photo on the left) and organic matter at confluence zone 7 (photo on the right) 92
Figure 6.2. Schematic to show how a changes from low (left schematic) to high flows (right schematic) causing hydraulic damming and back-up. Low flow channel-width is in dark black with high flow channel-width in dashed black. Arrows represent the water flow direction and thickness of the arrow represents level of discharge. Water can rise and fall within hours. During low flows, water flows out of the tributaries into in the main rivers and during high flows the main river can block the tributary flow or cause hydraulic damming. Sampling sites are highlighted as the river margin (R), river-stream interface (I) and upstream pool (U) above a high gradient riffle or waterfall where the main river only interacts during a significant storm event. The box in grey highlights the confluence zone. 94
Figure 6.3. Brunei is situated in the north of Borneo Island. The country is split into two parts, with Ulu Temburong National park within the Temburong district. Kuala Belalong Field Study Centre (KBFSC) is highlighted by the red dot and is situated on the banks of Sungai Belalong. The confluence zones included in this study are numbered 1-11 and identified by black dots. 96
Figure 6.4. Average AFDM of organic content separated into leaves, sticks/twigs and other/amorphous detritus at the confluence zone (river margin, river-stream interface and upstream pool). No statistical difference were found among these sampling locations. See text for standard deviation (river margin n= 11, confluence n = 8, tributary n = 11). 101

Figure 6.5. Bar plots with richness, average abundance and biomass (AFDM) of macroinvertebrates per kick sample at the river margin, river-stream interface and upstream pool. Error bars represent standard deviation (river margin n= 11, river-stream interface n = 8, upstream pool n = 11). 103
Figure 6.6. Bar plots with average abundance and biomass (AFDM) of shrimp per kick sample at the river margin, river-stream interface and upstream pool. Error bars represent standard deviation (river margin n= 11, river-stream interface n = 8, upstream pool n = 11).....104
Figure 6.7. Biomass of macroinvertebrates and shrimp with the main divide at 90+% can be explained by high abundances of shrimp. The letter represents the site; river margin (R), river-stream interface (I) and upstream pool (U), while the number is the tributary.....105
Figure 7.1. Brunei is situated in the north of Borneo. The country is split into two contiguous regions, with Ulu Temburong National park located within the Temburong district. Kuala Belalong Field Study Centre (KBFSC) and the study reaches including Lower Apan, Esu and Threelan (highlighted by asterisks) are all within the national park. 113
Figure 7.2. Extensive debris dam at a waterfall on Esu. The man indicates scale.....114
Figure 7.3. Percentage substrate (gravel, cobbles, boulders, and bedrock) of the three study streams (pooled across all biotopes) and of the four biotopes (pooled across all study reaches). 118
Figure. 7.4. Richness, density, and biomass (ash-free dry mass; AFDM) of macroinvertebrates for Apan Threelan, Esu, and Lower Apan as well as for each biotope (cascade, waterfall, riffle, and pool). Error bars represent standard deviations (Lower Apan, n = 14; Esu, n = 10; Apan Threelan, n = 11; waterfall, n = 6; cascade, n = 8; riffle, n = 5; and pool, n = 16). Taxa richness ($F = 9.02$, $p < 0.001$) and biomass ($F = 9.46$, $p < 0.001$) differed among tributaries. Taxa richness differed among biotopes ($F = 3.97$, $p < 0.05$). 120
Figure 7.5. Ordination of macroinvertebrate density among sampled biotopes. Environmental data were fitted to the ordination axis using the envfit function of the vegan package in R. The environmental factors displayed are those that are most strongly correlated with the taxa. This includes velocity, gravel (G), cobbles (C), and bedrock (BR; $p < 0.001$) as well as depth, small wood debris (SWD), and moss (M; $p < 0.05$). The biotopes are represented as symbols: pool, black triangle; riffle, black circle; cascade, white square; and waterfall, black star. List of taxa abbreviations are in the Appendix Chapter 7b (Stress = 0.16).....123
Figure 7.6. Dendrogram of the macroinvertebrate density of the biotopes. The first letters represent the study site and biotope type: E, Esu; T, Threelan; A, Lower Apan; C, cascade; RI, riffle; W, waterfall; and P, pool. Bold text is used to highlight fast flowing biotopes (i.e., cascades and waterfalls).....124
Figure 7.7. Sample-size-based rarefaction and extrapolation showing sampling effort was sufficient for each of the biotopes: pool, n = 16; riffle, n = 5; cascade, n = 8; and waterfall, n = 6. At each of the individual biotopes, three Surber sampling replicates were taken. ..128
Figure 8.1 Waterfall at Sungai Esu (photo on the left) and at Lower Apan (Photo on the right).....131
Figure 8.2. Map showing the location of Brunei Darussalam in northern Borneo and the Temburong District. The four study streams were Sungai Lower Apan, Sungai Baki, Sungai Threelan and Sungai Esu, which are highlighted along with the main rivers Sungai Belalong and Sungai Temburong 133
Figure 8.3. Bar plots showing the average density of fish, shrimp and macroinvertebrates. Macroinvertebrate density was estimated using Surber samples and shrimp data from

Surber and kick samples for each pool both above and below the waterfalls. Error bars represent standard errors (all below-waterfall pools, n = 8; all above-waterfall pools, n = 9; Sungai Apan Threelan, n = 6; Sungai Esu, n = 5; Sungai Lower Apan n = 6). No error bars are presented for fish density because all samples were combined in the field. There was a significant difference between above- and below-waterfall densities of fish ($p = 0.04$) and shrimp ($p \leq 0.01$), but no difference in macroinvertebrate densities ($p = 0.89$). There was also a significant difference in macroinvertebrate densities among the study streams ($p = 0.01$).....141
Figure 8.4. Leaf litter decomposition in pools above and below waterfalls (top) over 23 days in 2013 and (bottom) over 72 days in 2014. Differences were significant in leaf litter breakdown rates (k_d) among streams in 2013 over 23-day exposures ($p \leq 0.01$) and in 2014 over 26-day exposures ($p \leq 0.01$) and 71-day exposures ($p \leq 0.01$). In 2013, faster decay rates occurred over 23-day exposures below the waterfalls ($p \leq 0.01$), whilst in 2014, over 26-day exposures, decay rates were higher in pools above the waterfall ($p \leq 0.01$).....145
Figure 9.1. Hydropsychidae nets on a waterfall (left photo) and a close up image of a Hydropsychidae in its net (right photo).....153
Figure 9.2 Map showing the location of Brunei Darussalam in northern Borneo (A). Brunei Darussalam is split into two parts with this study being conducted in the Temburong district, the eastern section of Brunei (B). The twelve waterfalls are situated on tributaries off of the two main rivers Sungai Belalong and Sungai Temburong (C).....155
Figure. 9.3. Average percent contributions of stomach contents including amorphous detritus, animal tissue, CPOM, algae and fungal hyphae and Conidia in the five macroinvertebrates Hydropsychidae, Heptageniidae, Blephariceridae and Simuliidae.....159
Figure 9.4. Mean δ 13 C and δ 15 N values of basal resources leaf litter (LL) and periphyton (P) for the dominant macroinvertebrates found on the waterfalls in Ulu Temburong National Park. Macroinvertebrates include Buccinidae, Blephariceridae, Heptageniidae, Hydropsychidae and Simuliidae. Error bars indicate +1 SD.....161
Figure 9.5. Food web of the dominant macroinvertebrates present on rock based biotopes in Ulu Temburong National Park. Based on stomach contents analysis and stable isotope analysis. Line thickness represents contributions of the sources. 163
Figure 10.1 (Originally Figure 5.2) Photo of Sungai Belalong outside KBFSC. Photo on the left taken at 17.45 when the rain started and photo on the right taken at 20:00.....170
Figure 10.2. (Originally Figure 7.5) Ordination of macroinvertebrate density among sampled biotopes. Environmental data were fitted to the ordination axis using the envfit function of the vegan package in R. The environmental factors displayed are those that are most strongly correlated with the taxa. This includes velocity, gravel (G), cobbles (C), and bedrock (BR; $p < 0.001$) as well as depth, small wood debris (SWD), and moss (M; $p < 0.05$). The biotopes are represented as symbols: pool, black triangle; riffle, black circle; cascade, white square; and waterfall, black star. List of taxa abbreviations are in appendix 7b (Stress = 0.16) 172
Figure 10.3. Schematic displaying the influence of waterfalls on aquatic communities with fish dominated below-waterfall pools, while shrimp dominated above-waterfall pools. No difference in leaf litter (LL) decomposition between the above and below- waterfall pools but there was higher levels of periphyton in below- waterfall pools. Image of fish Susan Laurie Bourque © Canadian Museum of Nature and image of the shrimp is from the Food and Agriculture Organization ©. 173
Figure 10.4. (Originally Figure 9.4) Food web of the dominant macroinvertebrates present on rock based biotopes in Ulu Temburong National Park. Based on stomach contents

analysis and stable isotope analysis. Line thickness represents contributions of the sources. 175

List of Tables

Table 2.1. Flow type descriptions used to identify physical biotopes in the field (Newson and Newson, 2000) 36
Table 2.2. Functional habitats and their biological functions, adapted from Harper et al. (1995) 37
Table 4.1. Channel characteristics and physical conditions of the biotopes at stream 61a and at the tributary. Type C = cobbles, G = gravel, B = boulders and BR = bed rock 67
Table 4.2. SIMPER analysis of the top taxa contributing to the observed similarities between the biotopes. Summary results show the percentage contribution and the cumulative contribution of contributing taxa to each biotope with percentages. 72
Table 6.1. Channel characteristics and physical variables of the eleven confluence zones, one measurement was taken at the confluence site..... 99
Table 6.2. Physical measurements at the confluence zone. Including distance from the river margin to the river-stream interface and upstream pools, and the percentage substrate of bedrock (BR), cobbles (C), gravel (G) and silt & sand (S&S). There were no boulders in the confluence zones and there were no river-stream interface sites at confluence zones 6, 10 and 11 due to waterfalls. 100
Table 6.3. Periphyton growth rate (per day) and ambient periphyton at confluence zone's 1, 3, 8 and 10. 102
Table 6.4. SIMPER analysis of taxa contributing to the observed similarities between the biotopes. Summary results of the percentage contribution and the cumulative contribution of contributing taxa to each biotope. L= larvae 105
Table 7.1. Average physical conditions including depth, wetted and bankfull width, velocity, and discharge of the three study streams (pooled across all biotopes) and of the four biotopes (pooled across all study reaches). 117
Table 7.2. Percentage presence of functional habitats of the three study streams (pooled across all biotopes) and of the four biotopes (pooled across all study reaches). 118
Table 7.3. SIMPER analysis of the top taxa contributing to the study sites. Percentage contribution and the cumulative contribution of contributing taxa within each biotope are shown as percentages. L= larvae 121
Table 7.4. SIMPER analysis of the top taxa contributing to the observed similarities between the biotopes. The percentage contribution and the cumulative contribution of contributing taxa within each biotope are shown as percentages. L= larvae 122
Table 8.1. Average physical conditions of the pools at the four study sites including bankfull width, wetted width, average depth, average velocity and dominant substrate type. Dominant substrate type is given in percentage cover as G, gravel; BR, bedrock; B, boulders and C, cobbles. Pool substrate compositions significantly differed between above- and below-waterfall pools (chi-squared, Lower Apan $p \leq 0.001$; Baki $p \leq 0.001$; Esu $p \leq 0.001$; Threelan $p \leq 0.001$)..... 134
Table 8.2. Average leaf litter breakdown rates (k_d) for pools above and below waterfalls in 2013 (over 7 and 23 days) along with macroinvertebrate (both all and non-predacious) average richness, abundance and biomass (AFDM). Leaf litter breakdown rates significantly differed among streams in 2013 over 23-day exposures ($p \leq 0.01$). Higher leaf litter decay rates were also found in 2013 over 23-day exposures below the waterfalls ($p \leq 0.01$). There was no significant difference in macroinvertebrate biodiversity or shredder diversity between pools above and below the waterfalls..... 142

Table 8.3. Average leaf litter breakdown rates (k_d) for sites above and below waterfalls in 2014 (over 26 and 71 days) along with macroinvertebrate (both all and non-predacious) average richness, abundance and biomass (AFDM). Leaf litter breakdown rates differed among streams in 2014 over 26-day exposures ($p \leq 0.01$) and 71-day exposures ($p \leq 0.01$). There was also a significant difference in leaf litter breakdown rates between pools above and below waterfalls over 71-day exposures ($p \leq 0.01$). Over 71-day exposures, a higher abundance of macroinvertebrates accumulated in below-waterfall pools ($p = 0.002$). Higher non-predacious abundance occurred in pools below waterfalls ($p = 0.01$), whilst non-predacious invertebrate biomass was higher in above-waterfall pools ($p = 0.001$).....143
Table 8.4. Periphyton growth rates (per day) on the rocks in pools above and below waterfalls at the four streams in 2013 (23-day exposures) and 2014 (72-day exposures). Growth rates were higher below the waterfalls in 2014 ($p \leq 0.01$) but not in 2013 ($p = 0.3$). In 2014, there was also a difference in growth rates among streams ($p = 0.02$). 146
Table 8.5. Survey summary showing significant differences in animal communities and ecosystem functions among the study streams and between the above- and below-waterfall pools.....147
Table 9.1. Physical measurements of waterfalls including average slope angle and length. Presence and absence (1Vs 0) of functional habitats include leaf litter, wood debris and moss. ~ indicates estimated values.....158
Table 9.2. Average carbon and nitrogen isotopic values of basal food sources and consumers (mean + SD).....160

Acknowledgements

I thank my supervisor Dr. Michael Chadwick for 'planting the seed' of investigating and working in the streams of Ulu Temburong National Park. Throughout my project Mike's support and enthusiasm has been invaluable, from exploring my study streams (including swimming through snake invested pools!) to proof reading manuscripts and the final drafts of this thesis. I also thank my second supervisor, Prof. Nick Clifford for his supervision and support throughout my time at Kings.

Special thanks are extended to Rona McGill who introduced me to the world of stable isotope analysis at NERC Life Sciences Mass Spectrometry Facility in East Kilbride. While Katrin Layer-Dobra was super helpful in showing me the methods of gut contents analysis. Liam Yuk-Hon Butterfield was very kind to assist me in the lab to 'speed up' processing of the organic matter samples. I thank the technical support (along with tea and biscuits) provided by Trevor Blackall and Bruce Main in the lab at King's College London.

Identification of taxa would not have been possible without the assistance of Roy Whiles, Paul Clark, David Reid, Stephen Butler, Albert Orr, Manfred Jäch, Wagner Rüdiger, Virginija Podeniene, Marija Ivković, Herbert Zettel, Ignac Sivec, Ben Price, Michel Sartori and Jean-Luc Gattolliat. I very much appreciate their time in assisting with taxa identification.

This project would not have been possible without a Natural Environment Research Council (NERC) funded PhD studentship (Code: DAR7132) and NERC Life Sciences Mass Spectrometry (Code: EK232-11/14) for the stable isotope analysis. Thanks to the Universiti of Brunei Darussalam (UBD) and International Consortium of Universities for the Study of Biodiversity and the Environment (iCUBE) for fieldwork support and access to Kuala Belalong Field Study Centre (KBFSC). Logistically, this project would not have been viable without KBFSC and the hard working staff that keep the center alive. I thank my collaborators Rodzay Wahab and Rafhiah Kahar at UBD for assisting with logistics and export permits. Firdaus Ismail (undergraduate at UBD) kindly assisted me in the field in 2013 and 2014 and was a huge help when sampling the streams. While Aline Horwath, Hanyrol Ahmad Sah, Jacqueline Heckenhauer, Claas Damken and Lalit Dagar became very supportive colleagues and friends during my time in Brunei and when working at KBFSC for weeks at a time with no access to the outside world.

My time in the Department of Geography at King's College London has been enriching and enjoyable. I thank the Intrepid Explorers team, Briony Turner and Faith

Taylor, for the inspiring conversations and enjoyment of creating the Intrepid Explorer's platform. I thank the PhD community for being so welcoming and kind. I've been lucky to have gained lifelong friends during my PhD and at the risk of offending people, I'd like to say a special thanks to Arnout Van Soesbergen, Leo Zurita, Thomas Smith, Ben Smith, Samuel Tang, Sophie Blackburn, Daniel Schillereff, Mark de Jong, Anna Turbelin, Jake Simpson, David Smedley and Lara Langston.

I feel very privileged to have been given the opportunity to live my dream and work in the beautiful rainforests of Borneo. This would never have been possible without my parent's faith in my abilities and their understanding of the importance (and power) of education. I dedicate this thesis to the ultimate back-up team, Jean and Nigel Baker. Thank you.

Chapter 1: Introduction & Research Aims

1.1 Motivation and Context

The linkages between ecology, geomorphology and hydrology are responsible for eco-hydromorphic complexity through their shaping of river channels into networks of heterogeneous patches. Interactions between the hydrology, sediment dynamics and river morphology control the size, pattern and habitat structure of river channels (Church, 1992; Frissel, 1996). Eco-hydromorphology plays a crucial role in the spatial and temporal diversity of habitats, which are diverse, both within and among the many types of rivers. The classification and mapping of eco-hydraulic patch complexity within and among mesoscale habitats provides a robust technique for inferring how hydraulics, sediment dynamics and geomorphology influence stream habitats (Poff and Ward, 1990; Hart and Finelli, 1999; Bunn and Arthington, 2002). Eco-hydrogeomorphology has been a cornerstone of research within temperate stream ecology, and it has shown that flow regimes are a master variable in influencing the community structure of stream vertebrates and invertebrates (Stubbington *et al.*, 2009; Webb *et al.*, 2012). The central focus of this work, therefore, is to examine the eco-hydromorphic dynamics of tropical streams and the distribution and assemblages of benthic macroinvertebrates within the abiotic framework.

The tropics occur between latitudes 23°N and 23°S, and this equatorial region contains streams with high rates of insolation, warm waters and variable hydraulic regimes (Boulton *et al.*, 2008). Dobson *et al.* (2002) argued that tropical regions exhibit a complexity that goes beyond their waters simply having higher temperatures. For example, tropical streams lack seasonal inputs of leaf litter and have high variation in flow, creating an unpredictable detrital food source in many tropical reaches. Further, categorizing them just by location may not have much utility, as there is a wide variety of stream types, from headwater streams that drain high elevation, snow-topped mountains to those that slowly flow through lowland tropical forests (Ramirez *et al.*, 2008). Tropical streams contain a diverse range of plants and animals, with many creatures having unique adaptations to specific food sources, habitats or microhabitats (Ramirez *et al.,* 2008). However, taxonomic uncertainty has hampered investigations into tropical streams, with many species remaining to be described and identified (Jacobsen *et al.*, 2008).

The current understanding of tropical streams is relatively limited compared to that of temperate region streams. Most freshwater concepts or models have been developed in northwest Europe and North America regions that are quite distinct from tropical latitudes (Dudgeon, 2008). Dissimilarity in tropical geomorphology compared to temperate system geomorphology has been proposed in order to create a template for differences in ecological habitats (Boulton *et al.*, 2008). The location of streams and rivers near the equator and away from the polar ice caps makes these systems among the oldest, which has major biogeographical implications (Boulton *et al.*, 2008). It is important to understand the differences between temperate and tropical systems in regards to their geomorphology and ecology; ecosystem models based on temperate streams may not apply to tropical systems, and the management techniques used in temperate areas may be ineffective or even damaging to tropical systems (Boulton *et al.*, 2008).

The time frame for investigating and understanding basic tropical stream ecology is under pressure because of the strong anthropogenic impacts on these ecosystems (Dudgeon, 1995). The rate of degradation and loss of aquatic species is hampering our ability to understand pristine systems, as high levels of deforestation, land use change, high human population growth and pollution are occurring in many tropical stream catchments (Dudgeon, 1995; White *et al.*, 1998). Given environmental changes and associated losses of biodiversity, it is imperative to collect baseline data from natural pristine tropical systems and understand the processes and roles of these ecosystems. This information will further the current understanding of the potential consequences of extinction and declining biodiversity (Dudgeon *et al.*, 2006; Corlett, 2009). There are relatively few suitable places to examine natural ecosystem structure and function. For example, Borneo Island is home to one of the oldest rainforests in the world, at 130 million years old, and yet, over a relatively short time period, deforestation and land use change has largely destroyed this unique environment. A recent study conducted in northern Borneo (Fig 1.1) suggests that 80% of Malaysian Borneo has been heavily impacted by deforestation (Bryan *et al.*, 2013). However, neighbouring Brunei Darussalam, situated in northern Borneo, has been much more successful in preserving its natural landscape, with 54% of its land still covered in pristine rainforest (Bryan *et al.*, 2013). Therefore, Brunei Darussalam was the chosen location for this work.

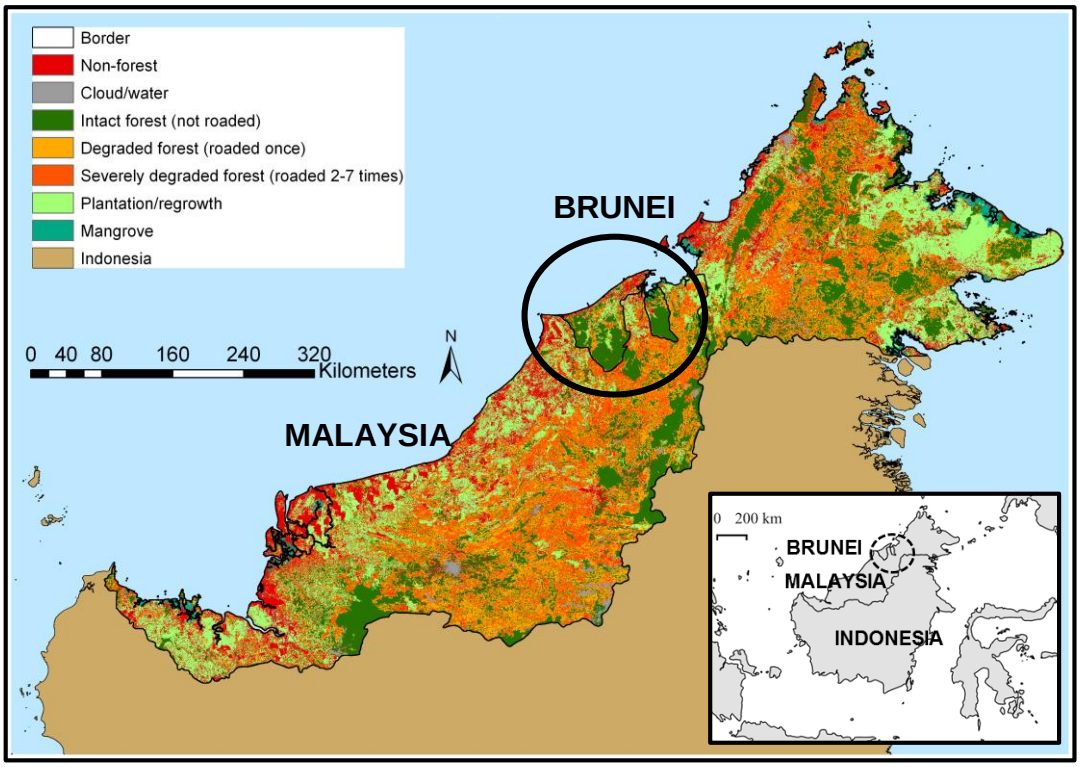


Figure 1.1. *Forest cover and condition in northern Borneo including Brunei, circled in black, and Malaysia as inferred from Landsat images in 2009. Indonesia is in brown. This image is adapted from Bryan (2013), with insert map showing the location of Brunei in Borneo Island.*

One way to explain the eco-hydraulic complexity described above is to investigate the structure and function of aquatic macroinvertebrates. Aquatic macroinvertebrates are small organisms that live for at least part of their lives in water, consisting mainly of insects but also including crustaceans, molluscs, leaches, oligochaetes and planarians. In pristine catchments, these creatures are essential to ecological services, functioning as the backbone of many food webs, vital to riverine ecology and serve as the primary diet for many fishes and mammals—macroinvertebrates are therefore crucial to understanding biodiversity patterns (Giller and Malmqvist, 1998). Tropical streams, such as those in Brunei, can be described as being composed of discrete units delineated by longitudinal boundaries of waterfalls and numerous small tributaries, which create ecological zonation for macroinvertebrates and other macroconsumers. This can contribute to various community patterns, evidence of which is demonstrated in this research. A significant difference between temperate and tropical streams, which may affect the biogeography of macroinvertebrates, is the frequent flood events that occur in the tropics, scouring out habitats and food resources.

1.2 Research Aims and Objectives

1.2.1 Overview

Owing to the scarcity of research in the tropics identifying truly representative eco-hydromorphic sites, it is not possible to adequately compare temperate and tropical streams. Linking and simplifying relationships between the physical variables and ecological responses is also not possible. Rather, this research explores theories and models developed in temperate systems to determine if they can be applied or appropriately adjusted to describe and explain some of the tropical stream processes. Classification concepts and theories from temperate streams are a reasonable starting point for investigating tropical systems. This ordering of information provides a systematic and clear method by which to view eco-hydrogeomorphology. This enables the interpretation of the variability and complexity that exists between many factors (Brierley and Fryirs, 2013). However, it is also clear that current concepts are insufficient; all models are a simplification of reality. Combining classification models may shed light on the distribution of macroinvertebrate communities; alternatively, a new or adapted scheme may well be required.

1.2.2 General Aims and Objectives

This PhD thesis focuses on the basic dynamics of pristine tropical stream systems. Brunei is used as a case study owing to its pristine rainforests and stream systems that have hitherto been little studied. The overall aim of this thesis is to develop an understanding of the basic dynamics of tropical stream systems in terms of eco-hydromorphology, with an emphasis on benthic macroinvertebrates in the context of temperate stream classification models and theories. The main objective of the research presented is therefore to improve understanding about eco-hydrogeomorphological tropical stream systems that have hitherto been little studied.

1.2.3 Research Questions and Hypotheses

More specific research questions that will be answered are listed below and numbered according to the chapter (C):

QC4. Are biotopes a useful classification tool for examining macroinvertebrate biodiversity in tropical streams?

QC5. Does macroinvertebrate diversity differ between the two main rivers of Ulu Temburong National Park, Sungai Temburong and Sungai Belalong?

QC6. Do organic matter (including leaf litter and wood debris) and levels of periphyton increase at the confluence? And does this create 'biodiversity hotspots' for macroinvertebrates at the confluences?

QC7. Are biotopes important, rather than streams or reaches, for the operational scale of tropical macroinvertebrate biodiversity?

QC8. To what extent do tropical waterfalls influence the distribution of aquatic communities? And will ecosystem functions (i.e. periphyton growth and leaf litter breakdown rates) differ between above- and below-waterfall pools?

QC9. What is the main source of food for tropical macroinvertebrates that live on waterfalls?

The main Hypotheses in this thesis are listed below and numbered according to the chapter (C):

HC4. Biotopes provide a useful classification tool for examining macroinvertebrate biodiversity in streams

HC5. Pool macroinvertebrate diversity would be lower in Sungai Temburong compared to Sungai Belalong due to its higher discharge and increased scouring flood events

HC6. There will be higher levels of organic material and periphyton in the confluence zone which will consequently increase diversity of macroinvertebrates

HC7. Biotopes, rather than streams or reaches, will be more important for the operational scale of biodiversity and thus be a useful classification tool for examining macroinvertebrate biodiversity in streams

HC8. Waterfalls will have a significant impact on fish densities, with lower numbers above waterfalls. While both shrimp and macroinvertebrates will occur in higher densities in the above-waterfall pools, which are lacking fish. Differences in community structure will influence ecosystem function including periphyton growth rates and leaf litter decomposition.

HC9. Due to the steep gradient of waterfalls and low retention of terrestrial based resources, the dominant basal food resources of macroinvertebrates would be algae

1.3 Research Methodology

Most of this thesis is based on survey data, rather than experimental manipulations, in order to describe the nature of the relationship between the stream biota (predominantly macroinvertebrates) and physical variables. Models are always simplifications that compromise complexity, relevance and realism. However, tropical systems are complex and filled with many interactions therefore to conceptualize and understand these processes, there is a need to reduce some of this complexity. There is an understanding of the limitations of survey data, rather than experimental, in that it is evidence for correlation, not causation.

1.4 Research Novelty

Tropical stream research is underrepresented in comparison to temperate stream research. Within tropical stream research there has been a focus on the Neotropics, Hong Kong and northern Australia, with very few studies on the pristine streams in Borneo. Level of research in Borneo is so limited that most aquatic life has not even been documented, with very little understanding of how macroinvertebrates may be distributed within the abiotic framework. Below is a list of outputs from this project:

- Journal papers accepted:
    - Eco-hydromorphic Classification for Understanding Stream Macroinvertebrate Biodiversity in Brunei Darussalam, Northern Borneo" Zoological Studies
    - "Fluvial biotopes influence macroinvertebrate biodiversity in Southeast Asian tropical streams" Ecosphere
    - "Benthic Community Structure and Ecosystem Functions in Above-and Below-Waterfall Pools in Borneo" Hydrobiologia
- Creation of a network of taxonomists (>25) that specialise on tropical macroinvertebrates to assist with identification of tropical macroinvertebrates. This has resulted in the confirmation of numerous first recordings of macroinvertebrates. Taxa have been donated to the Natural History Museum in London, Natural History Museum in Lausanne (Switzerland), Oxford University of Natural History and Universität Kassel (Germany). Donated taxa have been used to assist with the development of identification keys, DNA analysis and to help increase the understanding of their life histories.

- Education document of the most common macroinvertebrates found in Ulu Temburong National Park. Given to Kuala Belalong Field Study Centre (KBFSC) to be used with undergraduate students and school children when studying the streams
- Film of tropical macroinvertebrates in my study streams 'Life in streams and waterfalls, Borneo': goo.gl/oqVP3a
- Photos of macroinvertebrates in my study sites: flickr.com/photos/tropical-streams

1.5 Research Challenges

- Conducting fieldwork in remote tropical regions brought many challenges. Fieldwork took place in two locations in Ulu Temburong National Park. The first location was near Bukit Pagon, which was accessible by helicopter. A temporary base camp was constructed that consisted of a string of tents. The limited facilities included electricity from a generator to power and charge equipment in the evenings. The second location was Kuala Belalong Field Study Centre (KBFSC), where most of this research was conducted. KBFSC is situated 30–60 minutes from the nearest inhabited area by long boat (depending on water levels) and is a 3-4 hour journey to the Bandar Seri Begawan. There is no Internet and limited mobile signal at the field study centre. A generator powers electricity for a few hours in the morning and evening. Conducting fieldwork in remote regions can impact safety. Below are some of the main challenges I faced and ways in which I reduced any risk:
    - Attended a Royal Geographical Society (RGS) first aid course that specialized in geographical fieldwork to ensure I have the skills to deal with any medical problems
    - Fieldwork was always conducted with a local boat man or research assistant
    - Life jackets were worn when in the water
    - During storm events water levels can rise significantly (and very fast) making fieldwork unsafe. Fieldwork would finish for the day when there were any signs of water levels rising or when it started to rain
    - The base camp for the Pagon research trip was located near the Malaysian boarder where illegal loggers are known to be located. Therefore Universiti Brunei Darussalam ensured that all researchers were accompanied by armed guards during fieldwork

- Permits for the exportation and importation of macroinvertebrate samples often took longer than expected
- Poor knowledge of tropical macroinvertebrates with many larval macroinvertebrates not yet described past family level. To assist with identifications I used special camera equipment to photograph the macroinvertebrates and uploaded onto the Flickr website (http://flickr.com/photos/tropical-streams/sets/), where interested experts could comment or request to see the actual specimens. I visited the Natural History Museum in Lausanne and the Zoological department of South Wales to work on identifications.

1.6 Thesis Outline

This thesis is organised as cumulative work, in the form of a general introduction and conclusion along with the six empirical chapters which include three accepted peer review journal papers and one manuscript which is currently under review (Fig 1.2). As a result of this structure, each empirical chapter can be read independently with its own introduction, methods, results and discussion. Consequently for the whole thesis reader this creates repetition particularly of the introduction, context, site descriptions and method sections. The remaining chapters in this thesis are organised as follows:-

Chapter 2: Literature Review

An overview of the current literature is provided in Chapter 2. The first section of this chapter introduces macroinvertebrates and their importance in river systems. The second section provides an overview of eco-hydromorphology in the temperate environment including general background about the subject and an assessment of the importance of eco-hydromorphology classification models. The third section of the literature reviews the state of tropical stream research.

Chapter 3: Background to Research Sites in Ulu Temburong National Park

Brunei Darussalam is described in Chapter 3, with an overview of the climate, landform processes and the rivers and streams of Ulu Temburong National Park, including a summary of the main study sites.

Chapter 4: Eco-hydromorphic Classification for Understanding Tropical Stream Macroinvertebrate Biodiversity

Eco-hydrogeomorphology and macroinvertebrate biodiversity of two remote, tropical streams in northern Borneo are examined in Chapter 4. This study was part of a larger project that gathered experts from around the world to conduct the first systematic study of the environment and biodiversity of the high altitude primary rainforest of Bukit Pagon.

Chapter 5: Macroinvertebrate Diversity in Pools of Sungai Temburong & Sungai Belalong: A Short Communication

The distribution and diversity of macroinvertebrates in the two main rivers that flow through Ulu Temburong National Park are described in Chapter 5. This study was conducted along Sungai Temburong and its tributary, Sungai Belalong. It attempts to answer the research question "is the distribution what we would expect?"

Chapter 6: The Effect of River–Tributary Interactions on Benthic Habitat & Macroinvertebrate Biodiversity

To date most research on confluences has focused on the effect of tributaries on the main rivers; by contrast there have been very few studies on how the main rivers affect their tributaries. Therefore this chapter investigates quantities of organic matter (including leaf litter and wood debris) and periphyton (biomass and growth), along with macroinvertebrates in 11 confluence zones that experience hydraulic damming/reverse flows.

Chapter 7: Fluvial biotopes influence macroinvertebrate biodiversity in Southeast Asian tropical streams

The effects of biotopes on macroinvertebrate community structure in three streams within Ulu Temburong National Park are characterized in Chapter 6. Biotopes within these streams were categorized as waterfalls, cascades, riffles or pools. It examines the question as to whether these set of biotopes are good candidates to characterize macroinvertebrate community structure.

Chapter 8: Benthic Community Structure & Ecosystem Functions in Above- and Below-Waterfall Pools in Borneo

The stream landscape is characterised by the many waterfalls and poses the research question how does this affect benthic community structure and ecosystem function. A systematic survey and analysis of aquatic communities, litter decomposition and periphyton growth rates for above- and below-waterfall pools in four tributaries of Sungai Temburong and Sungai Belalong is examined and presented in Chapter 8.

Chapter 9: Macroinvertebrate Trophic Structure on Waterfalls in Borneo

Stable isotope analysis (using $\delta^{13}C$ and $\delta^{15}N$ of leaf litter and periphyton) and gut contents analysis are presented in Chapter 9. This analysis was conducted to investigate the trophic structure of dominant macroinvertebrates living on waterfalls.

Chapter 10: Conclusions

The final and concluding chapter brings together the main findings of the thesis and determines the extent to which the research objectives have been met. This chapter also provides recommendations for future research.

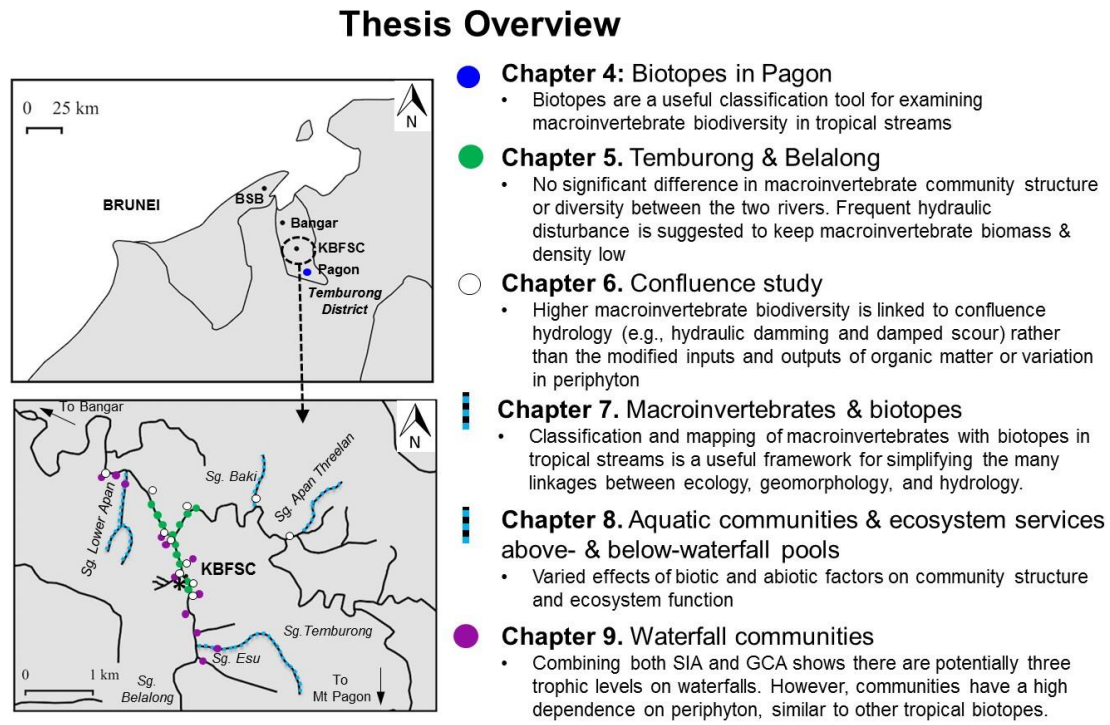


Figure 1.2 *Thesis overview and summary from each data chapter*

Chapter 2: Literature Review

General Introduction

In order to understand macroinvertebrate diversity and distribution patterns in tropical streams, it is essential to review previous studies. This literature review is split into three sections. The first section provides an introduction to macroinvertebrates and their importance in river systems. The second section reviews eco-hydromorphology in the temperate environment, including general background on the subject and an assessment of the importance of eco-hydromorphology classification models, and both statistical and conceptual treatments. The third section briefly reviews tropical stream research.

2.1 Introduction to macroinvertebrates

Macroinvertebrates are the foundation of riverine ecology and are integral to the diets of many fish and mammals (Giller and Malmqvist, 1997; Wotton, 2001). These creatures support numerous ecosystem functions, including the breakdown of organic matter (e.g. leaf litter, wood debris and seeds), while aquatic invertebrates such as Stoneflies shred and consume the material (Wotton, 2001). Other aquatic invertebrates, including flattened mayflies and snails, consume periphytic algae and biofilms, helping to control growth and transfer energy up the food chain (Gessner *et al.,* 1999; Wotton, 2001). Filter feeders, including black fly larvae, consume suspended organic matter, thus reducing turbidity, and in turn excrete faecal pellets, which is a more accessible form of organic matter for other macroinvertebrates to consume and can fertilize algae and aquatic plants (Wotton, 1994; Joyce *et al.*, 2007). Macroinvertebrates are also a useful tool for monitoring biodiversity patterns because their distributions often reflect patterns of food resources and habitats (Ramirez *et al.*, 1998). Similarly, their sensitivity to changes in benthic resources and pollution events makes them especially useful in biomonitoring, thus providing insight into environmental changes (Giller and Malmqvist, 1997; Everaert *et al.*, 2014). Accordingly, it is important to understand how macroinvertebrates utilize habitats within the channel.

2.2 Introduction to eco-hydromorphology

The first known study of the interaction between organisms and their environment was conducted in 1869 by Ernst Haeckel, who is considered by many to be the founder of ecology (Begon *et al.,* 2006). Haeckel stressed that the biota is shaped by environmental factors and interactions, therefore closely linking ecology with evolutionary theory. However, it took many decades before this thinking was fully integrated into riverine science, a relatively new discipline that originated in Europe during the early 20th century. The first papers connecting ecology and the physical landscapes of rivers were by German scientists—Steinmann (1907; 1908), who studied insects, and Hora (1922), who worked with fish. However, it was not until a few decades later that the general notion of "eco-hydraulics" was devised by Hino (1977), a Japanese researcher who proposed this term to capture the influence of aquatic plants on their streams. Hino pushed for hydraulic science to go beyond its contemporary boundaries as a discipline and include ecology (Kundzewicz, 2002). Zalewski (2002) describes ecohydrology as the 'third phase' in the development of ecology, with the first being descriptive and the second seeking to understand processes as well as seeking to control and manipulate ecological processes.

Whilst the numbers of published interdisciplinary articles and books has increased, there have been semantic arguments over the name of this potentially new discipline and whether it should be classed as such (Kundzewicz, 2002). It has been argued that truly interdisciplinary research conducted by geomorphologists and biologists is still rare (Bond, 2003; Hannah *et al.*, 2004; Hannah *et al.*, 2007; Rice *et al.,* 2010). Hannah (2004) argues that the terms hydro-ecology used by hydrologists and eco-hydrology by ecologists highlight this divide. Lancaster and Downes (2010) find these semantic arguments about the definitions of eco-hydrology or hydro-ecology pointless. The prefix 'eco' implies ecological, and when it modifies the root word 'hydrology', the term describes the interaction between ecological and hydraulic processes. Vaughan *et al.* (2009) proposed that the linking of interrelated disciplines (i.e. hydrology, geomorphology and ecology) should be considered eco-hydromorphology. This research makes use of the latter definition, which is formally defined by Vaughan *et al.* (2009, p114) as "the interactions of the biological entities and ecological processes of a river with the hydrological and geomorphological form and dynamics".

This confusion over linking ecology, hydrology and geomorphology is perhaps reflected by the lack of understanding of the eco-hydromorphic mechanisms, and this is

highlighted by failed river restoration projects. Palmer *et al.* (1997) posited the "Fields of Dreams" hypothesis in reference to the many projects that are largely disconnected from ecological theory and have too strong a focus on geomorphology. There is limited empirical evidence to demonstrate that an increase in channel heterogeneity promotes biological recovery, and yet it is still a major restoration technique (Palmer *et al.*, 2010). Rice *et al.* (2010) emphasized this lack of scientific understanding and questioned the dubious benefits of one of the most widely adopted river restoration tools, the use of physical structures to increase river heterogeneity. Some studies have found that placing wood or boulders in a reach can increase the number of ecological habitats (Riley and Fausch, 1995; Roni and Quinn, 2001), but others found no real change or benefits (Olson and West, 1989) and some failed completely (Frissell and Nawa, 1992). This inconsistency demonstrates the real disconnect between the physical and ecological aspects and theory (Palmer *et al.,* 1997; Lake *et al.,* 2007).

Within the eco-hydromorphology literature, the terms 'mechanisms' and 'functions' are repeatedly used, but often without an explicit definition. As these scientific terms are critical to the meaning of the research, it is important to define them. A function that is performed by an ecosystem is a result of interactions involving organisms and their habitats (Lecerf and Richardson, 2009). In the literature, the term function has been defined in terms of river properties and services. According to Lecerf and Richardson (2009), 71% of studies on aquatic ecosystem functions deal with litter decomposition and consumer performances in small, heterotrophic streams. According to Dunham and Beaupre (1998, p30) a mechanism is "an appropriate level of reductionism that provides a causal explanation of the functional relationship among a set of variables". The relationship between the pattern and mechanism is scale dependent and hierarchical in nature. Models have been designed, primarily from empirical research on temperate streams and rivers, to classify ecological or geomorphological processes, but more recently they have been developed to integrate eco-hydromorphology at different spatial scales. The next section discusses stream classification models, including both statistical and conceptual models.

2.3 Stream classification model research

2.3.1 Introduction

O'Keeffe *et al.* (1994) have argued that classification helps to organize and explain complex objects, systems and ideas. As classification models are used to simplify the current

knowledge and are probabilistic representations of ecosystem classes, they can be treated as hypotheses (Goodwin, 1999). These can then be used as a framework to investigate tropical systems. Stream classification models have attempted to simplify the complex interactions and functions of fluvial systems (Fig 2.1). Historically, most scientific disciplines undergo a phase of classification with the objective of ordering observations (Gordon *et al.*, 2004). For river management, classification simplifies the system to enable tasks that would otherwise be impractical, including habitat inventories, assessing anthropogenic impacts and judging stream quality against legislative requirements (Gordon *et al.,* 2004).

The appeal of classification models is that the concepts can be understood by non-specialists, through simplified language and relatively easy to understand methods (Gordon *et al.*, 2004). These characteristics are attractive for an inter-disciplinary research area, such as eco-hydromorphology, because the ideas and connections are collated into a format that can be understood by all players. There are two types of stream classification models: statistical and conceptual. Statistical models use large data sets in an attempt to find relationships between species occurrences and environmental characteristics in fluvial systems, implicitly emphasizing the current ecological conditions of organisms. Statistical models tend to be restricted within geographic regions, but they allow predictive suites of physical variables or characters to identify species assemblages (Ross, 2013). In contrast, conceptual models use the general features of habitats to make predictions about the ecological traits of assemblages or species. These models can be especially powerful when predicting the effects of disturbances on faunas and ecosystems (Ross, 2013).

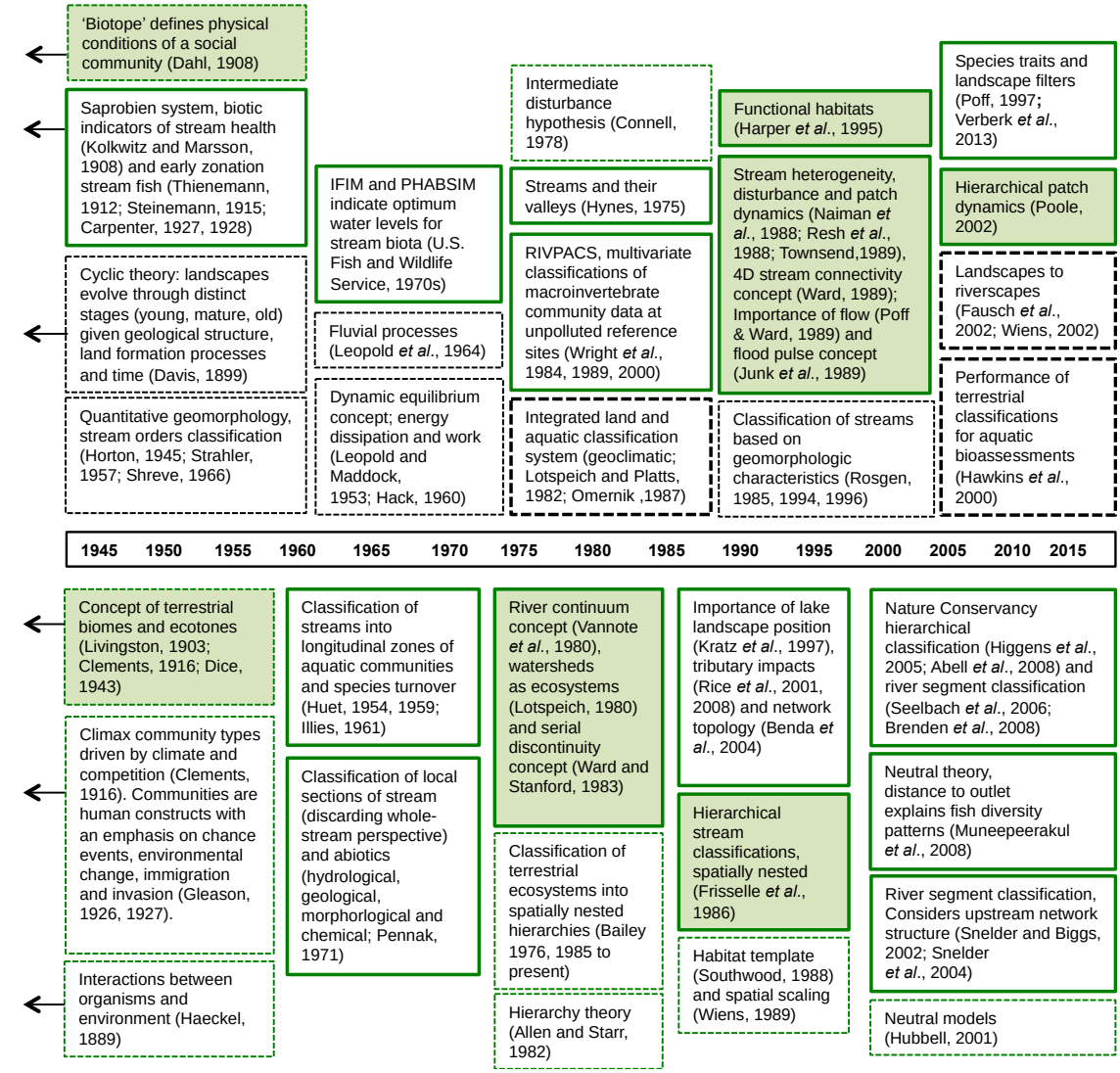


Figure 2.1. *Timeline of the key theories and contributions to stream eco-hydromorphology, with a focus on aquatic classification. These concepts are not fixed in time, but usually develop, overlap and intermingle with other ideas over time. The outline of each box indicates the field in which the developments occurred: stream geomorphology (thin, dashed black line), ecology (dashed green line), stream ecology (thick green line), and integrated terrestrial-aquatic systems (thick, dashed black line). The shaded green boxes indicate aquatic classifications that this thesis focuses on. (Adapted from Melles et al., 2012).*

2.3.2 Key statistical models

IFIM and PHABSIM - One of the first management techniques to integrate the hydrology and ecology of a fluvial system is the instream flow incremental methodology (IFIM), which was developed with the physical habitat simulation (PHABSIM) software in the 1970s. It was originally designed to determine optimum water levels for fish and was eventually applied to benthic invertebrates (Gore, 1978; Gore and Judy Jr., 1981), but this

was not judged to be successful because of the difficulties in collection, taxonomic identification and developing habitat suitability curves. Armitage (1989) and other researchers designed the river invertebrate prediction and classification system (RIV-PACS) to predict changes in macroinvertebrate species composition caused by changes in environmental conditions. This system uses a combination of chemical water quality measurements, hydraulic conditions and geomorphology to estimate predictive relationships through an approach that is based on the multivariate TWINSPAN analysis (Hill, 1979). These models are based on abundance–environment relations that describe the densities of individuals relative to the physical variables of the environment. There has been criticism raised against the PHABSIM model for its lack of ecological accuracy; like many hydraulic habitat analyses, it does not represent within-reach scale variability in habitat availability.

Biotope and functional habitat statistical research - Biotope theory is based on the influence of current ecological conditions on organisms. Under this framework, the organisms currently living in a habitat match the present day habitat conditions; therefore, although these organisms are shaped by historical events and phylogenetic relationships, they are not entirely constrained by them (Townsend and Hildrew, 1994). The German scientist Dahl (1908) devised the term "biotope" to define the physical conditions of a social community ("Biocönos") in which plants and animals are interdependent. The difference between habitat and biotope concepts has been debated over the decades, with 'biotope' referring to the abiotic environment of a community and 'habitat' referring to the abiotic environment of a species (Udvardy, 1959; Whittaker *et al.,* 1973; Price, 1975; Ward, 1992).

In river systems, biotopes can be observed as river surface flow features (i.e. flow biotopes) such as riffles, pools, runs and cascades, each of which reflect combinations of sediment, depth and velocity associated with the structure of the river bed (i.e. physical biotope). The generally accepted flow type (or flow biotope) biotope classification scheme as advocated by Newson and Newson (2000) is shown in Table 2.1. The two main factors that support and define the biotope concept are measurements using the Froude number and the relationship between channel morphology and surface flow (Jowett 1993 and Wadeson, 1994; Clifford *et al.,* 2006). The Froude number (Fr) is a dimensionless index used to classify water flow. It is essentially a ratio of inertial forces caused by friction that impede water flow to the gravitational force that moves water down a gradient (Giller and Malmqvist, 1998). For Fr values of less than 1.0, water flow is slow and calm, whereas for Fr values of

greater than 1.0, flow is fast and turbulent. This hydraulic variable is important because it is related to water surface roughness (Statzner *et al.*, 1988), which is the main biotope identification characteristic used in the field.

Management approaches over the past 20 years have increasingly stressed the need to integrate the biological and physical aspects of riverine environments (Clifford *et al.*, 2006). A promising alternative approach emerged in the late 1990s with the identification and characterization of physical biotopes and the functional habitat (*sensu* Harper *et al.*, 1995) classification scheme (Padmore, 1998; Table 2.2). Functional habitats describe types of stream habitat based on substrate and vegetation cover (Newson and Newson, 2000). As Clifford *et al.* (2006) pointed out, the appealing aspect of the biotope approach is its practicality in the field and ability to capture river complexity in a way that sufficiently characterizes riverine environments. These types of rapid assessment models are useful tools for assessing river complexity and diversity. In the UK, biotopes have been used as part of the River Habitat Survey (RHS). Although 'physical biotope' and 'functional habitat' approaches are derived from different disciplines, they are complimentary and have the potential to integrate physical and biological research in rivers.

Harvey *et al.* (2008) argued that combining the functional habitat classification approach with biotopes can provide a new and distinct ecological classification scheme. Initial attempts to combine these approaches have failed; however, Clifford *et al.* (2006) noticed that specific functional habitats appear to be associated with several flow biotopes, which they suggest indicates that an amalgamation or reduction of flow types is necessary. Harvey *et al.* (2008) analysed RHS data to investigate whether the functional habitat classification scheme could be statistically associated with biotopes to create a new distinct ecological classification scheme. The conclusion was that if the biotopes were amalgamated into three predominate biotopes, then there was a statistical association between the habitat classes, but no distinct relationships among all biotopes. Accordingly, combinations of the predominant biotopes may more accurately represent flow conditions. However, ecological distributions are not solely influenced by geomorphology; other factors, such as different dispersal characteristics for plants and general biotic interactions, can affect the compatibility of biotopes and functional habitats (Harvey *et al.*, 2008).

Table 2.1. *Flow type descriptions used to identify physical biotopes in the field (Newson and Newson, 2000)*

FLOW BIOTOPE	DESCRIPTION	ASSOCIATED PHYSICAL BIOTOPE
Free fall	Water falls vertically and without obstruction from a distinct feature, generally more than 1 m high and often across the full channel width	**Waterfall**
Chute	Fast, turbulent flow over boulders or bedrock with a smooth boundary; flow is in contact with the substrate, and exhibits upstream convergence and downstream divergence	**Spill** (chute flow over areas of exposed bedrock) **Cascade** (chute flow over individual boulders)
Broken standing waves	White-water 'tumbling' waves with crests facing in an upstream direction; associated with 'surging' flow	**Cascade** (at the downstream side of the boulder flow diverges or 'breaks', rapid)
Unbroken standing waves	Undular standing waves in which the crests face upstream without 'breaking'	**Riffle**
Rippled	Surface turbulence does not produce waves, but symmetrical ripples that move in a general downstream direction	**Run**
Upwelling	Secondary flow cells visible at the water surface as vertical 'boils' or circular horizontal eddies	**Boil**
Smooth boundary turbulent	Flow in which relative roughness is sufficiently low that very little surface turbulence occurs; very small turbulent flows cells are visible, reflections are distorted and surface 'foam' moves in a downstream direction; a stick placed vertically into the flow creates an upstream-facing 'V'	**Glide**
Scarcely perceptible flow	Surface foam appears to be stationary and reflections are not distorted; a stick placed on the water's surface will remain still	**Pool** (occupies the full channel width) **Marginal deadwater** (does not occupy the full channel width)

Table 2.2. *Functional habitats and their biological functions, adapted from Harper et al. (1995)*

Type	Functional Habitat	Biological Functions
Substrate and substrate grain size	Exposed rock & boulders	'Hygropetric zone', a thin film of water on the surface of bare rock provides habitat for small macroinvertebrates
	Cobbles/ gravel	Hyporheic zone within interstitial spaces, ideal fish spawning site, supports invertebrates
	Sand	Usually species-poor, but can support large numbers of smaller, specialized invertebrates; accumulation around obstructions such as woody debris can become more stable and biologically richer.
	Silt	Silted pools provide a detritus-rich habitat for macroinvertebrates that is stable for much of the summer.
Habitats based on the morphological characteristics of aquatic plants	Trailing vegetation/ Marginal plants	Feeding, mating and oviposition zone for mature stages of otherwise aquatic invertebrates.
	Emergent macrophytes	Passage to the surface for emerging insects, attachment surface for filter-feeders and oviposition sites
	Floating-leaved plants	Passage to the surface for emerging insects
	Submerged, broadleaved	Smooth surfaces for attachment & protection from fast flows and predators
	Submerged, fine-leaved	Protection from predation and turbulence; provides surface for periphyton growth and attachment of invertebrates
	Mosses	Oviposition in faster flowing waters Protection from predation and flow; accumulation of fine sediment and organic matter provides physical substrate and food
	Macroalgae	Food source for grazers, case material for some chironomids, refuge from predation and oviposition sites
Habitats based on riparian terrestrial vegetation	Leaf litter	Direct food source for shredders, site for production and capture of fine particulate organic matter and can act as an extension of the physical substrate; particularly important in finer sediments
	Wood debris	Direct use by borers; increases channel stability
	Tree roots	May provide important habitat for specialized and rare species, but undersampled

Despite the difficulty in integrating the physical biotope and functional habitat inventories within investigations of aquatic invertebrates, this approach can still be a framework that enables investigation in tropical systems. The biotope has been used as a sampling framework for some tropical river studies, including Furtado (1969) and St Quentin (1973) in Malaysia and Sri Lanka, respectively, to investigate Odonata assemblages. Other studies have used the pool and riffle biotopes to assess assemblages longitudinally in tropical

systems (Bishop, 1973; Rundle *et al.,* 1993). However, there have been few if any tropical studies that have investigated the structure, composition and pattern of biotopes in terms of macroinvertebrate assemblages and functional habitats. Functional habitats occur at the microscale, which is associated with small organism microhabitats and influenced by flow hydraulics that can cause changes over the course of seconds (Fig 2.2). The mesoscale corresponding to structures such as pools and riffles (i.e. various biotopes) are stable over slightly longer time periods. Both the microscale and mesoscale are part of an organized hierarchy in which the higher, broader macroscale systems (i.e. drainage basins and stream systems) impose constraints on the lower order systems (Frissel *et al.,* 1986).

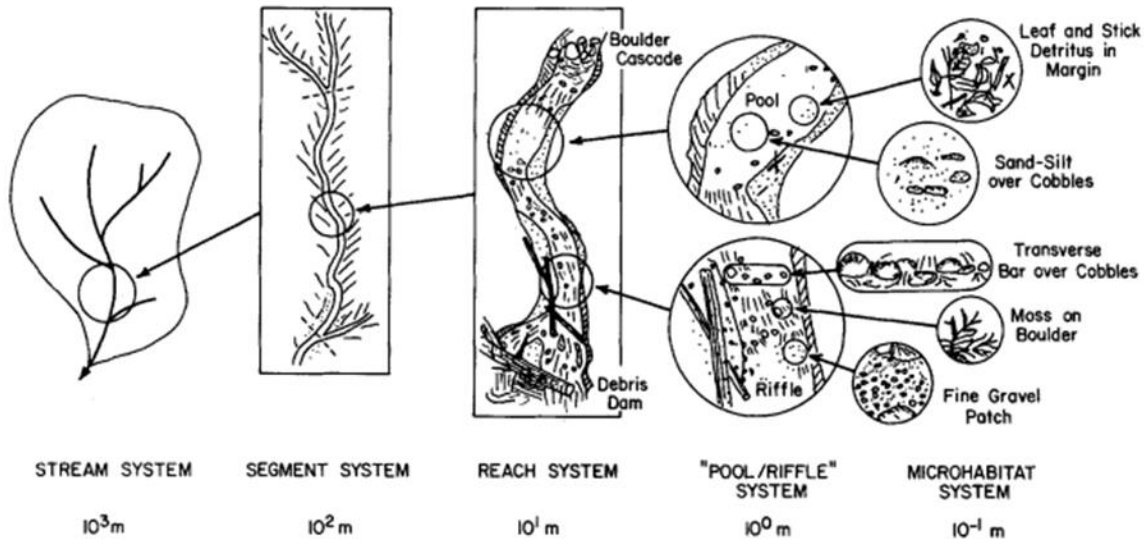


Figure 2.2. *View of streams in a hierarchical context. In this stream system and associated habitat subsystem, the microhabitats are part of the microscale, while pools and riffles along with reaches are part of the meso-scale and the drainage basin constitutes the macroscale (Adapted from Frissel et al., 1986).*

In the literature, there has been a general focus on pool and riffle biotopes, with less work on other biotopes, such as waterfalls (Clayton 1995; Rackemann *et al.*, 2013; Clayton and Pearson, 2016). Waterfalls are distinct from other biotopes because they lack a hyporheic zone and a water column of sufficient depth for the normally abundant shrimp and herbivorous fish, leaving highly specialized macroinvertebrates to dominate the biotope. Although waterfalls only represent a small part of the length of rivers, their specialized (and significantly under-researched) biota and functional habitats make them ecologically important areas that require special protection (Pearson, 2016). Waterfalls may be of

particular interest in tropical streams as longitudinal blocks for fish movement, creating upstream refugia for taxa including shrimp and tadpoles (Covich *et al*., 2009*;* Hein and Crowl, 2010), but also in their own right as a potentially more stable biotope type because of their bedrock substrates and consistent flows.

2.3.3 Key Conceptual based models

Habitat template model - In 1977, Southwood created the concept of the habitat template model, a framework that provides a predictive pattern for the evolutionary assembly of communities and life history traits (Ross, 2013). It emphasizes the association of the species with habitat features, so that species occur where they are best suited. This approach assumes that the present day ecological traits of organisms match the current ecological conditions. Townsend and Hildrew (1994) developed testable predictions of the habitat template model, for both species traits (e.g. age, size, reproductive type, movement, etc.) and assemblage traits (e.g. biotic interactions). They focused on temporal habitat heterogeneity and spatial heterogeneity to predict the response of species traits or assemblage traits to habitat templates (Fig 2.3). Two axes were used in their analysis, temporal heterogeneity, a measure of the frequency of disturbances, and habitat heterogeneity, a measure of the abundance of refugia. As disturbances increase in frequency, the number or accessibility of refugia become increasingly important. A more heterogeneous template would therefore be an indicator of a more frequently disturbed system. Predictions from the habitat template model determine that assemblage traits would also be influenced by the frequency of disturbance, with shorter life spans and smaller body sizes being more typical of organisms in an unstable system. In a more stable system, assemblages would be more specialized in terms of resource use, with a greater importance of biotic interactions.

This phylogenetically informed approach was tested on the upper reaches of the River Rhône, in which 13 animal and plant taxonomic groups were analysed, but it was ultimately concluded that the spatio-temporal variability did not form a template for the species traits (Resh *et al.,* 1994). The relationships between organisms and variability were therefore predicted to be more complex (Menezes *et al.,* 2010). However Southwood's hypothesis inspired many models such as that of Vannote *et al.* (1980), i.e. the river continuum concept (RCC), and trait-based approaches.

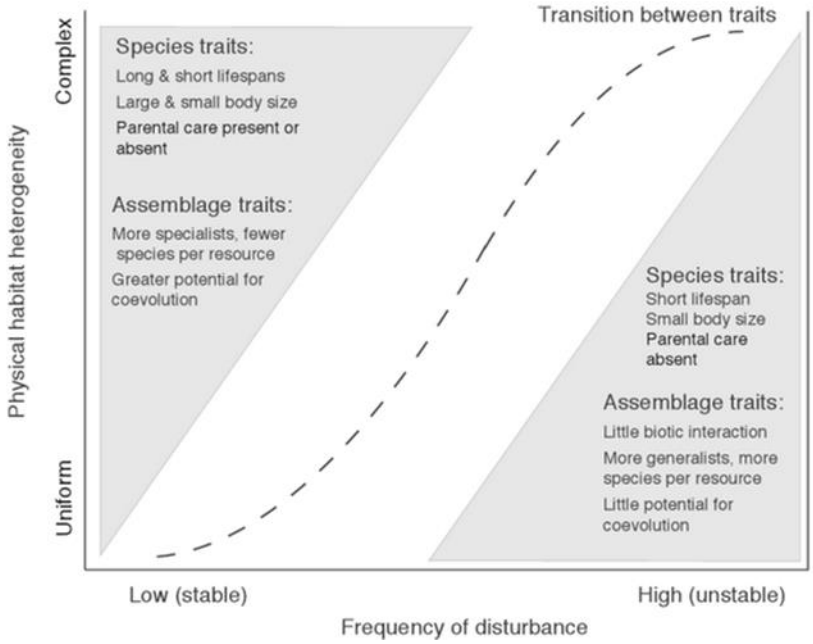


Figure 2.3. *Habitat template model showing the relationship of assemblage traits or species to the frequency of disturbance and complexity of the physical habitat. The shaded areas represent the different predicted traits and dashed line shows the transition point. Diagram from Ross (2013), which was adapted from Resh et al. (1994) and Townsend and Hildrew (1994).*

River continuum concept - A major focus of stream ecology for the past 30 years has been the investigation of longitudinal patterns that regulate both biodiversity and ecosystem functions. The RCC, since it was developed by Vannote *et al.* (1980), has become one of the most influential frameworks for ecological research in the last century. The RCC constitutes a holistic analysis of energy flow in river systems, under the hypothesis that the structure and functions of biotic stream communities adapt to conform to the mean state of the physical system. Thus producer and consumer communities reach equilibria with the physical conditions of the channel, and as the physical conditions of the stream change downstream, the biological communities also change (Fig 2.4; Vannote *et al.*, 1980). According to the RCC, a river channel is divided into three main sections. First, the headwaters are dominated by allochthonous material from riparian vegetation, and high levels of shredders consume this detritus; the production to respiration ratio is low (<1) in headwaters. Second, mid-reach channels are less dominated by riparian vegetation, and increased light yields an increase of autochthonous material. Grazers and collectors increase in mid-reach channels, with the former eating the periphytic algae and the latter collecting fine organic particulate matter (FOPM) flowing in from upstream. Third, in the lowland reaches, there is little influence from riparian vegetation, and the water is too turbid for

primary production to occur. In lowland reaches, collectors dominate, feeding off the FOPM from upstream, and the primary production to respiration ratio is again low (<1). Predators and microbes are relatively constant throughout the river.

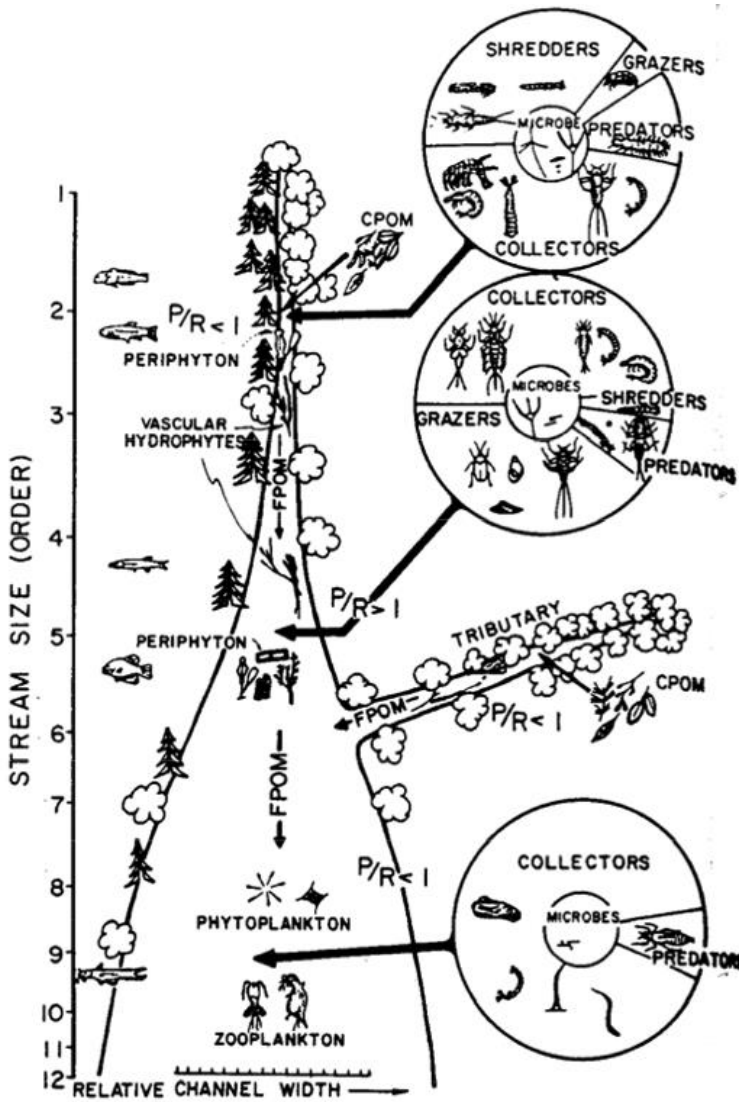


Figure 2.4. *Original diagram from Vannote et al (1980) illustrating the river continuum concept. The river channel is divided into three main sections.*

The theory is based on research conducted in temperate regions of the USA, and there have been many discussions of its relevance in a worldwide context (Winterbourn *et al*., 1981; Minshall *et al*., 1985; Junk *et al*., 1989). Despite disagreement over its worldwide applicability, this approach has successfully sparked debate among ecologists and encouraged a more holistic approach to riverine science. From the relevant studies that have been conducted (e.g. Sehal, 1983; Sivaramakrishnan and Venkataraman, 1990; Rundle *et al*., 1993;

Ormerod *et al.*, 1994), it has become apparent that changes do occur in zoobenthos assemblages along the continuum, especially across a significant altitudinal range. However, many of these studies have been conducted in systems that are influenced by pollution or other anthropogenic factors (Dudgeon, 1996a). Even fewer studies have examined at whether the River Continuum Concept can be applied to tropical rivers. Greathouse *et al.* (2005) tested the RCC in Puerto Rico, finding agreement with the RCC model in terms of predators, scrappers and shredders, but collectors and filterers exhibited a pattern that contradicts the RCC.

River discontinuity concepts - The serial discontinuity concept (SDC) proposed by Ward and Stanford (1983) began with the RCC and questioned what would happen to the patterns and processes if a weir or dam were placed in the river. This concept mainly makes predictions about the nature of energy and materials, rather than about specific organisms. Interestingly, despite this approach being widely cited, few field studies have applied it. The river discontinuum concept argues that no specific river is a continuum, with rivers being separated into discrete parts (Poole, 2002). Many natural habitat fragmentations can create discontinuities in the continuum, including waterfalls and high gradient riffles. Waterfalls can be natural barriers that limit the migration of fish and create areas of refuge for shrimp and crabs in reaches above waterfalls (Covich *et al.*, 2009; Hein and Crowl, 2010; Karssing *et al.*, 2012; El-Sabaawi *et al.*, 2015). The different community structures above and below waterfalls can influence a range of ecosystem functions, including organic matter decomposition and periphyton growth (Greathouse and Pringle, 2006; Moulton *et al.*, 2010; Ho and Dudgeon, 2016).

Confluences are another cause of discontinuities. Where tributaries join the main river or where two tributaries of similar sizes combine, gaps are created in the downstream succession of habitats (Rice *et al.*, 2001; Church, 2002). Vannote *et al.* (1980) describes confluences as potential sites of continuum disturbances because downstream habitat succession discretely changes to a new trajectory as the streams abruptly increase in size. Rice *et al.* (2001) highlights the impact of tributaries on the main stem river, creating step-like adjustments that change channel width and depth, substrate type and size, organic material, nutrients, water and temperature, all of which ultimately affect biota. Confluences have been described as biodiversity hotspots owing to the higher diversity of their benthic communities (McGuire and Winemiller, 1998: Rice *et al.*, 2001; Knispel and Castella, 2003). However, there

has been a lack of research on the effects of main river stems on biodiversity in tributaries, with the two exceptions conducted in temperate regions including a Beckmann *et al.* (2005) study on the Rhine and a Wilson and McTammany (2014) study on the Susquehanna River (USA). In tropical stream catchments, localized storms can significantly increase discharge in some parts of the network, which causes the main river to increase in discharge, producing frequent hydraulic damming or even 'back up' in back channels or tributaries (Herrera and Rondon, 1985). This potentially frequent change in the hydrology of tributaries can influence macroinvertebrate communities, yet there have been no relevant studies conducted in tropical streams.

Species traits and landscape filters - The species traits and landscape filters approach provides a heuristic framework for understanding and predicting the composition of local biological communities (Poff, 1997). This approach may move stream ecology away from being a descriptive science and towards being a predictive science, with species' traits indicating the causal mechanisms and relationships between species and the environment (Verberk *et al.,* 2013). The species traits and landscape filters approach is based on the logic that habitats with similar environments impose similar selective forces, requiring species to have similar attributes or adaptations to survive (Poff, 1997). Poff (1997) documented the example of shredder distributions, which are dependent on riparian vegetation; even when microhabitat conditions are ideal, these species require coarse leaf litter.

However, problems with this approach have been identified, including different representations of species traits when comparing assemblages across environmental gradients (Statzner *et al.*, 1994; Townsend *et al.*, 1997). In addition, there is an inconsistent relationship between traits and environmental conditions (Statzner and Bêche, 2010). Verberk *et al.* (2013) argue that the two main problems that have arisen with this approach are the low discriminatory power of the traits and the poor mechanistic understanding it provides. These weaknesses are caused by poor knowledge of the most important traits for environments and an inability to account for combinations of traits to create functional equivalents. A species traits approach would be problematic if implemented in tropical streams because knowledge of macroinvertebrate functions and life histories are especially poorly understood in the tropics. Poff *et al.* (2006) suggest focusing on traits that are uncorrelated with phylogeny (such as size, voltinism and trophic habitat), as these have a direct relationship with stressors, therefore linking traits directly to the current environment.

Fluvial landscape ecology at the patch scale - According to Forman (1995, p39) a patch is a "relatively homogeneous non-linear area that differs from its surroundings". Pringle *et al.* (1988) emphasized that temporal and spatial scales change with context, for example, with the organism being studied or question being asked. The patch for a salmonid may include much of a river, but a Simuliidae patch may only cover a rock during its larval stage. Patch approaches differ from biotope theory as the concept places importance on the patch hierarchy, arrangement, structure and juxtaposition; Poole (2002) defined this as the metastructure. The metastructure is important in determining the function and behaviour of patches (Poole, 2002); if patches are re-arranged along a river's course, this would impact the ecological dynamics of the system, even if the relative proportions of patches are unchanged (Fisher *et al.,* 1998). Biodiversity may even be higher at the often heterogeneous patch boundaries (Ward and Weins, 2001).

The framework for integrating patch mosaics has been defined as the hierarchical patch dynamics view (HPD), which frames the fluvial system as nested and discontinuous patch mosaics (Fig 2.5; Poole, 2002). One benefit of this structure is its ability to include the various river dimensions; according to Ward (1989), there are four, including the lateral, longitudinal, vertical, and temporal dimensions (Fig 2.5). The temporal scale is especially important, as rivers are constantly adjusting with season, climate and changes in the catchment. This framework views rivers as part of the landscape, not as separate entities, which is important when attempting to gain a holistic understanding of rivers.

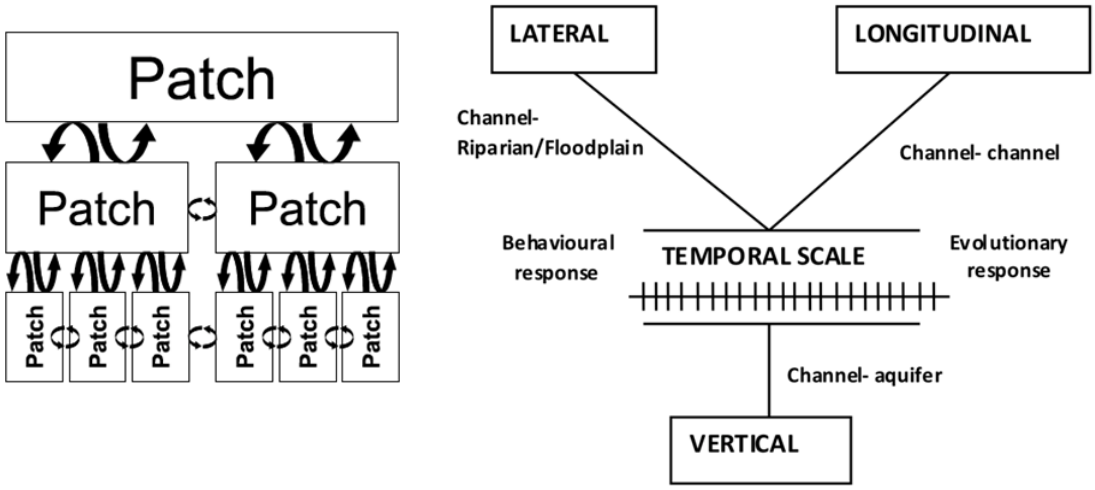


Figure 2.5. *Hierarchical patch dynamics view (HPD), which conceptualizes the fluvial system as nested and discontinuous patch mosaics (on the left), and the four dimensions of a river modified from Ward (1989; on the right).*

2.4 Tropical Stream research

Tropical stream research is underrepresented compared to temperate stream research; for example, 94% of studies published in the Journal of Freshwater Biology were conducted in North America, Europe, Australia and New Zealand (Hildrew and Townsend, 2007). Although in recent years there has been a drive to increase the geographic representation of stream research, the tropical regions continue to be underrepresented (Dudgeon, 2015). The schematic in Fig 2.6 illustrates some of the main developments in tropical stream research and those that are most relevant to this thesis. Overall, few tropical stream studies have focused on macroinvertebrate distribution with regard to hydro-geomorphology (Fig 2.6). Research in this area is limited by taxonomic uncertainty, which is caused by a lack of identification guides and consequent difficulties for non-specialists to identify tropical species (Jacobsen *et al.*, 2008). Accordingly, Jacobsen *et al.* (2008) have argued that to improve understanding of macroinvertebrate ecology in tropical streams, taxa should be identified to the family or sub-family levels, and then morphotyped. Significant insight into ecosystem functions can still be gained at this limited identification level.

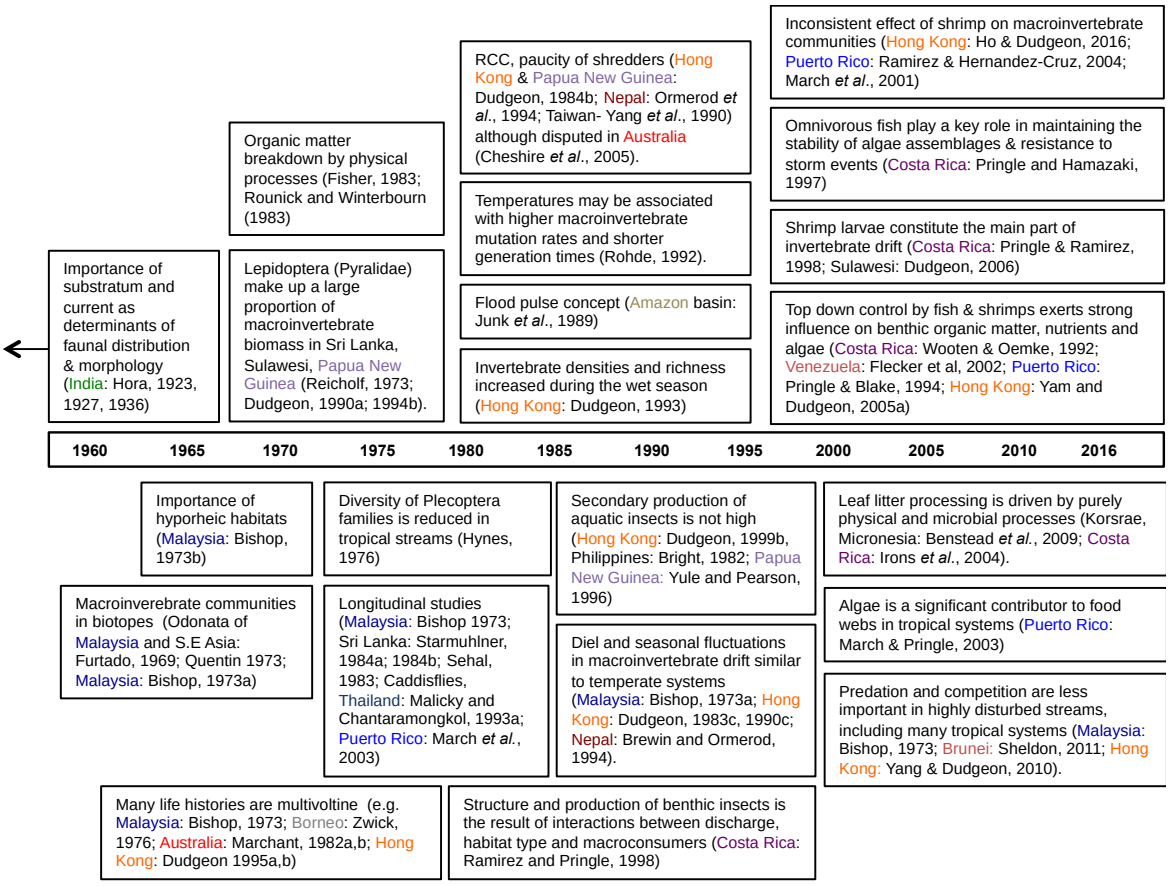


Figure 2.6. *Key tropical studies with a focus on benthic macroinvertebrates; studies have been dominated by those conducted in a few countries and regions. Countries are highlighted in various colours.*

Benthic communities are rather different in tropical streams relative to other biomes with more substantial herbivorous fish, shrimp and tadpole populations (i.e. macroconsumers; Power, 1984; Flecker, 1992). Amphibians in tropical streams are more diverse and have been shown to directly influence the availability of algal resources, with tadpoles being classed as ecosystem engineers, modifying benthic habitats for other species (Flecker and Taylor, 2004). Pringle and Hamazaki (1997) found that the natural assemblages of omnivorous fish control periphyton levels and reduce the impact of frequent high-discharge events. Grazers play a very important role in clearing deposited sediments from benthic substrates after flood events (Power, 1984; Flecker, 1992). Without grazing communities, sediment deposition would cover benthic surfaces, which would limit light availability to the lower strata of periphyton. In low-nutrient systems, which include many tropical rainforest streams, macroinvertebrate and macroconsumer excrement may also be a source of nutrients for the periphyton.

A hierarchy of factors can affect macroinvertebrate populations, including water chemistry (Everaert *et al.*, 2014), supply of functional habitats (Harper *et al.,* 1995) and macroconsumers (Pringle and Hamazaki 1998). However, Dudgeon *et al.* (1993) argue that flood events reset benthic densities. In many tropical regions, intense rainfall frequently occurs in the late afternoon, causing water levels to rise dramatically over a brief period of time. Flood events can cause scouring of functional habitats (including leaf litter and algae) and the movement of sediments, thus reshaping the channel and influencing low flow biotopes and habitats. These regular precipitation events can affect animal populations by direct physical removal or by indirectly washing out in-stream functional habitats (Bond and Downes, 2000). However, unlike in other biomes, the tropics are aseasonal forests; therefore, trees shed their leaves throughout the year, providing a continuous supply of allochthonous material. Bishop (1973) suggests that these constant but unstable functional habitats combined with non-seasonal life cycles suppress macroinvertebrate population densities. Traditionally, flooding is considered a disturbance event, but in frequently flooded river systems, perhaps it should not be classed as a disturbance. By using some of the eco-hydromorphology classification models described in this chapter, this thesis attempts to increase the current understanding of how macroinvertebrates negotiate habitats in tropical streams.

2.5 Summary

Most of this thesis is based on survey data, rather than experimental manipulations, in order to describe the nature of the relationship between the stream biota (predominantly macroinvertebrates) and physical variables. The author understands the limitations of these surveys and the specific limitations of data based on species densities without taking into account population densities, which are determined by vital rates, including those of births, deaths and migration (Anderson *et al.*, 2006; Lancaster and Downes, 2010; Rice *et al.*, 2010). However, as Lamouroux *et al.* (2010) point out, although a better understanding of the complex biophysical interactions underpinning the abundance–environment relationships is necessary, models are always simplifications that compromise complexity, relevance and realism. Tropical systems are complex and filled with many interactions; in order to conceptualize and understand these processes, there is a need to reduce some of this complexity. There is an understanding of the limitations of abundance–environment

relationships, i.e. they are evidence for correlation, not causation. Accordingly, this thesis uses both statistical and conceptual models to analyse the stream systems of Ulu Temburong National Park. Biotopes in particular provide a physical framework to sample macroinvertebrate communities; this is an appealing classification system because of its simplicity, permitting identification by observing surface water features. This allows the discontinuity concept to be investigated, while assessing the effect of tributaries and waterfalls on functional habitats and macroinvertebrate communities.

Chapter 3: Background to Research Sites in Ulu Temburong National Park

3.1 General Introduction

This chapter describes Brunei Darussalam with a focus on Ulu Temburong National Park, where the research presented in this thesis was conducted. Fieldwork was carried out at two locations in Brunei Darussalam, in streams surrounding Bukit Pagon and Kuala Belalong Field Study Centre (KBFSC). This chapter provides an overview of the climate and landform processes as well as an introduction to the rivers and streams of Ulu Temburong National Park, including a description of the main study sites.

3.2 Introduction to Brunei

Brunei Darussalam is an oil rich microstate in the northwest of Borneo Island, covering an area of 5,765 km^2 (Fig 3.1). The country has an abundance of natural areas, with 59% and 22% of its area covered in primary rainforest and secondary forests, respectively (Bryan, 2013). Its total population is 392,000, of which 25% is rural, with most people living along the coastal urban areas (FAO, 2014). Fieldwork was conducted in the Temburong district (1,288 km^2) of Brunei, which is physically separated from the rest of Brunei by a narrow region of Sarawak, Malaysia (Fig 3.1). Most of the Temburong district is covered in primary rainforest and the Southern part is composed of Ulu Temburong National Park, with few if any Bruneians inhabiting the reserve (Cranbrook and Edwards, 1994). The region is a roadless and unlogged catchment (Sheldon, 2011), making the area increasingly unusual in Borneo. This thesis project is focused on streams draining Mt Pagon and those around Kuala Belalong Field Study Centre (KBFSC). Mt Pagon is situated in a very remote location, with access possible by helicopter or through many days trekking; therefore, only two stream reaches in this remote area were studied. KBFSC is more accessible, at 30–60 minutes from the nearest inhabited area by long boat depending on water levels; therefore, most fieldwork was conducted in this area.

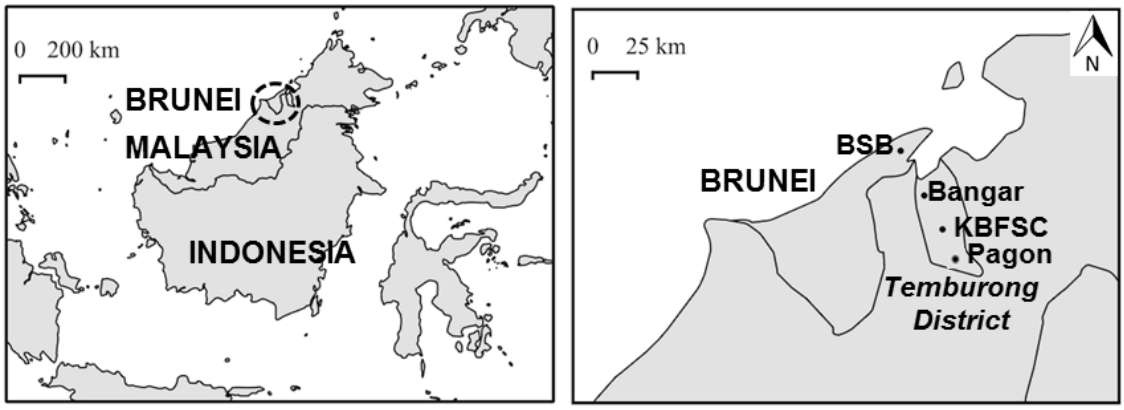


Figure 3.1. *Borneo Island is divided into three countries, Indonesia, Malaysia and Brunei (on the left). Brunei Darussalam is shown; note that the country is split into two parts with Sarawak (Malaysia) separating the Temburong district from the rest of the country (on the right). The capital, Bandar Seri Begawan, is situated in the larger section of Brunei. Bangar is the largest town in the Temburong district.*

3.3 Climate in Brunei and Ulu Temburong National Park

The humid tropics, such as Borneo, are characterized by frequent and intense storms that cause fluctuating river levels. Brunei has a tropical climate, influenced by the monsoon seasons of Southeast Asia (Dykes, 1996). During November, the intertropical convergence zone (ITCZ) moves south across the equator to sit above Indonesia; this causes Brunei to experience north-easterly winds from December to March, defined as the Northeast Monsoon (Dykes, 1996). The Southwest Monsoon occurs over Brunei from June to September as the ITCZ moves north across the Equator causing southeast trade winds to be dominant in Brunei. As the direction of the monsoon winds are almost parallel with the coast, this causes rainfall seasonality that is apparent in long-term rainfall data, which shows peaks in May and November (coinciding with the ITCZ movement over Brunei; Fig 3.2). Annual rainfall is also influenced by altitude, with precipitation rising from around 2,300 mm per year on the coast to over 4,000 mm annually in the tropical rainforest mountains (Cranbrook and Edwards, 1994). Despite the annual climate pattern coinciding with ITCZ movement, daily weather is often very erratic (Cranbrook and Edwards, 1994). This is even more pronounced in the rainforest, and Dykes (1997) argues that no month can be considered "dry" in Brunei, with every month of the year averaging over 200 mm of rainfall [based on monthly rainfall recordings from Semabat (1973–1991) and KBFSC (1991–1992)]. Dykes (2000) states that the Belalong area is particularly wet, even relative to world rainforest levels, with 240 rain days per year.

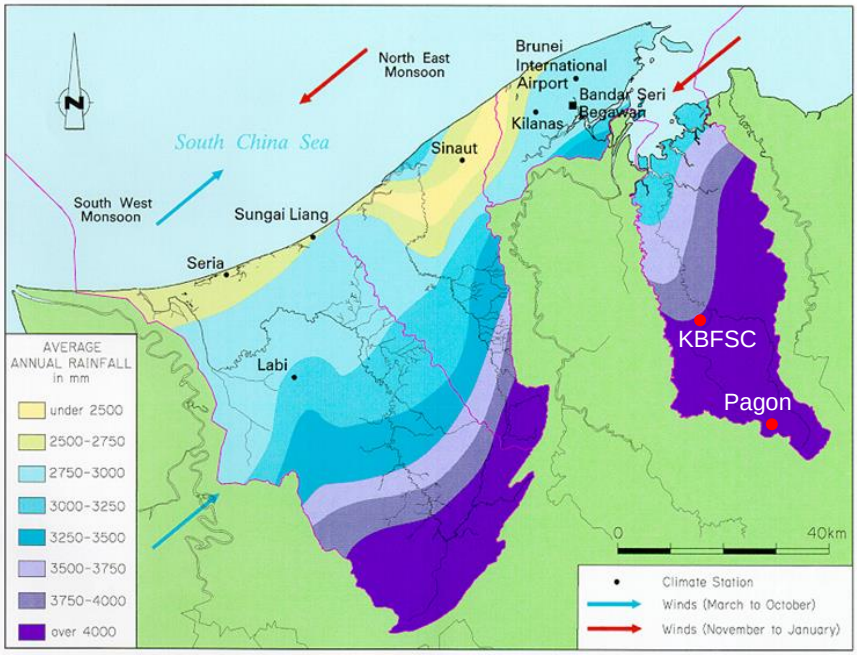


Figure 3.2. *Average rainfall and seasonal wind directions caused by the movement of the intertropical convergence zone. This image is from the Curriculum Development Department, Ministry of Education, Brunei Darussalam. The author has added the location of the two fieldwork sites, Kuala Belalong Field Study Centre (KBFSC) and the base camp for the Bukit Pagon expedition.*

Rainfall patterns around Belalong tend to be dominated and caused by thunderstorms, with warm, moist air rising to create dense clouds. Several convection cells may develop together with their condensation causing heavy rain. Often these storm events can be very localized; during the Brunei Rainforest Project (1991–1992) led by the Royal Geographical Society and Universiti Brunei Darussalam, researchers recorded on the 18th of November 1991, 63.5 mm of rain fall at KBFSC, but only 19 mm less than 10 km away at Bukit Belalong (Cranbrook and Edwards, 1994). The typical intensity profile of storms is characterized by most rain falling within approximately one hour. The most intense storm recorded during the Brunei Rainforest Project (1991–1992) was on the 19th of September 1991 when 116.5 mm of rain fell in 75 minutes (Cranbrook and Edwards, 1994). Although most rainfall events lack the power to move large rocks and change biotope structures, these events do transport significant amounts of bedload and scour epilithic algae (Sheldon, 2011). Fine layers of sediment are then deposited on rocks, coinciding with the falling limb of hydrographs; it is unclear whether the removal of this sediment is then caused by grazing fishes (Sheldon, 2011). Significant reductions in temperature occur during storm events, and although not all rainfall events are accompanied by such reductions in temperature, Dykes

(2000) found that a fall of 4°C in an hour is not uncommon. This can be explained by the heat energy from the air being dissipated through evaporation of rainwater from the wet forest canopy while light levels also decrease during storm events.

On the coast, the average annual temperature is 27°C and tends to be slightly lower during the wet season because of increased cloud cover. Daily temperatures range from 22°C just before dawn to 32°C in the early afternoon (Cranbrook and Edwards, 1994). Around Belalong, during the Brunei Rainforest Project (1991–1992) the highest temperature recorded was 37.1°C, whereas the average for most months was about 26°C. Relative humidity at Kuala Belalong at night is normally between 98% and 100% (Cranbrook and Edwards, 1994), whilst the minimum daily relative humidity generally occurred during the early afternoon, coinciding with the highest temperatures (Dykes, 2000). During the field research period (i.e. 2012–2014), average temperatures were relatively consistent at the KBFSC weather centre, apart from high temperatures at the end of 2013, when there was a peak in temperature that coincided with a decrease in total precipitation (Fig 3.3). Total monthly rainfall fluctuated over the months, with a general pattern of higher precipitation during November and April to May.

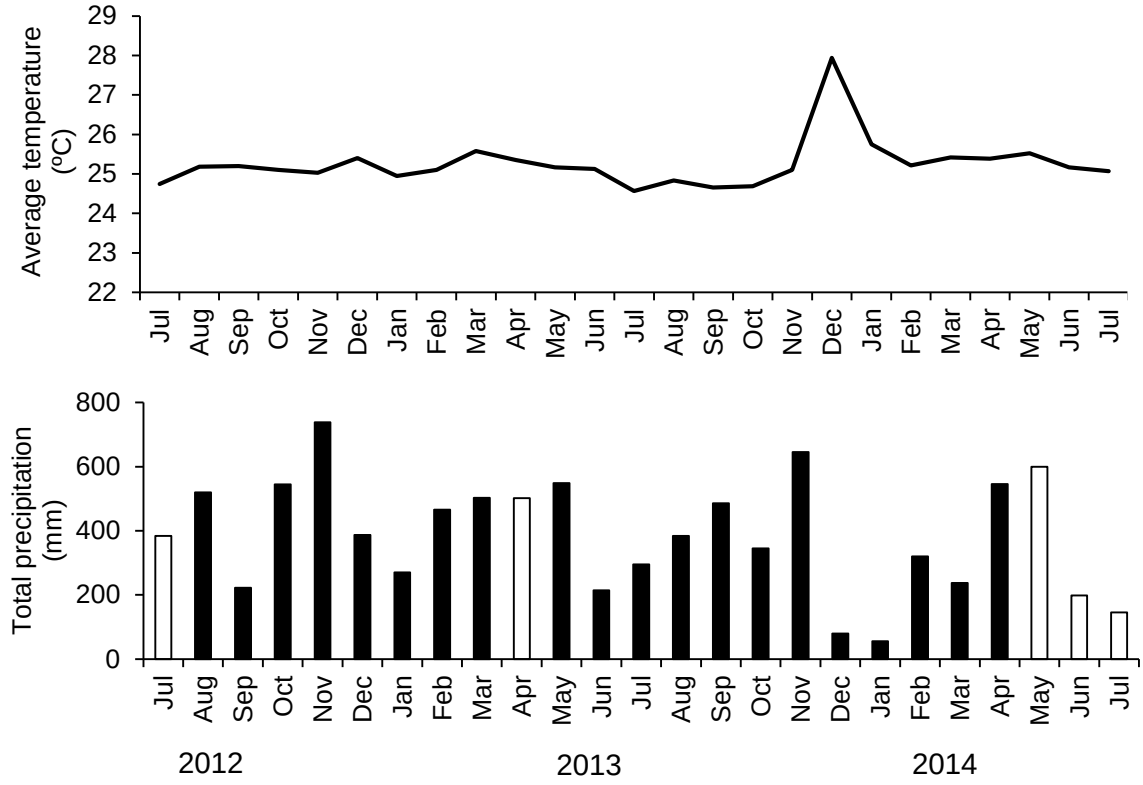


Figure 3.3. *Average temperature (top graph) and total precipitation (bottom graph) recorded at the weather centre at Kuala Belalong Field Study Centre. Bars in white indicate when fieldwork was conducted.*

3.4 Landform processes in Ulu Temburong National Park

Brunei is situated in a geological zone where eroded sediments from an older landmass to the south were deposited off shore from an old coastline that was to the southeast of the current coastline (Dykes, 1994). The present coastline of northern Borneo has been posited to be a former continental margin, consistent with the geology of the Ulu Temburong National Park, which is composed of sedimentary rocks, laid down approximately 16–30 million years ago as deltaic and marine deposits (Dykes, 1994). Subsequently, the area has been folded as the apparent result of an uplift that took place slowly but continuously, at a rate of 20 mm per century over the past two million years (Dykes, 1994). Erosional processes that have occurred with this uplift have produced a sharp topography characterized by a high degree of relief. The bedrock is composed of steeply dipping bedding planes, which influences the shape and steepness of the valley slopes and ridges (Dykes, 1994). Owing to the geological structure in the southeast of the Temburong district, the drainage pattern of local rivers all flow north westerly, with generally short tributaries joining from each side, forming a trellis pattern (Dykes, 1994).

The landscape of Ulu Temburong National Park is dramatic, with KBFSC at 30 m above sea level and Bukit Pagon, the highest mountain in Brunei, rising to 1,850 m above sea level. Belalong reaches 913 m above sea level, with the stream network travelling down the mountain to Kuala Belalong over a relatively short distance. The topography is composed of deep V-shaped valleys with no floodplains. The slopes and channels are coupled, which causes the sediment, water and nutrients to be conveyed straight from the surrounding catchment to the stream system (Brierley and Fryirs, 2008). High waterfalls are common along the tributaries and some smaller channels may only flow during rainfall events. Climate affects the physical landscape, with soil erosion and landslides being the dominant geomorphological processes that follow rainfall (Dykes, 2000). The rate of weathering and soil production is higher than the rate of long-term transport by water and gravity, which is the case in many humid tropical regions; this causes accumulation of organic material on the hillslopes (Dykes, 1994). There are two types of landslides that occur at Belalong, shallow and deep seated, with the later often involving an entire section of hillslope (Dykes, 1994; Fig 3.4A). Landslides have many important ecological implications; a pulse of sediment to the rivers and streams changes the channel geomorphology, increases turbidity and adds

nutrients to the otherwise nutrient-poor system (Fig 3.4B). Landslides also create gaps in the forest, allowing light to penetrate down to the streams.

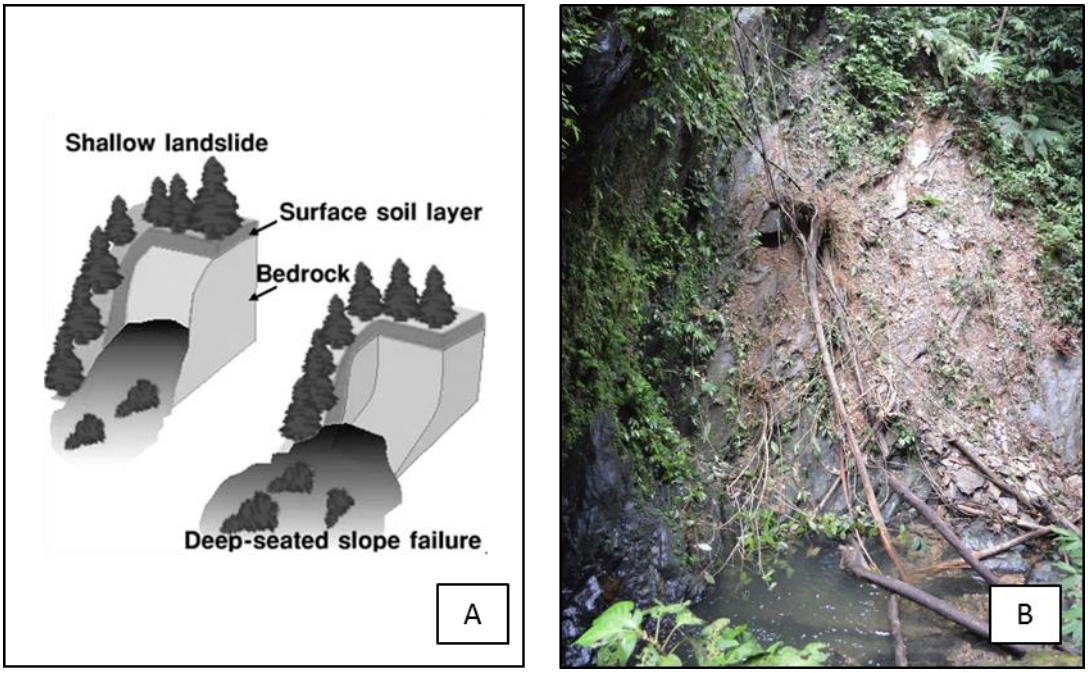


Figure 3.4. *(A) Schematic illustrating shallow and deep-seated landslides. (Image from Thinktheearth.net) (B) Shallow landslide that occurred along Sungai Esu in 2014.*

3.5 Rivers and streams of Ulu Temburong National Park

3.5.1 Geomorphology and physical factors

The Sungai Temburong drainage basin occupies 1,100 km^2 and is the third largest catchment in Brunei. Geomorphic features such as waterfalls, channel slopes and pool depths develop over time, shaped by the hydrological power, weathering and faulting (Covich *et al.*, 2009). They have created a habitat template for the flora and fauna as well as a hierarchical organization that can change the paths of migratory species under different flow conditions (Covich *et al.*, 2009). It is important to understand these landscape-level dynamics for the management of benthic species because of the complexity of stream ecosystems and assembly of food webs (Covich *et al.*, 2006; Jackson and Fureder, 2006). The rivers in the Temburong catchment incise into the uplifted landmass headwaters from the sea; this progression is indicated by the presence of waterfalls (Dykes, 1994; Fig 3.5). Today, waterfalls are present along the smaller tributaries, which have the lowest erosive capacity, with few tributaries incising all the way to the headwaters. Another feature of an incising channel is the regular supply of sediment, which can only be transported during the highest floods (Dykes, 1994). This sediment eventually accumulates at the confluence as tributaries

supply too much for the main river to transport; this protects channel beds from the rivers' energy in causing downward incision (Dykes, 1994). Most boulders and pebbles in the catchment are fresh (dark grey) or weathered (orange/pink) shale with some grey-yellow sandstone that has been transported from the headwaters in the southeast. Debris dams and single tree trunks are also common in the streams, creating in channel habitats and heterogeneity of flow, with pools and sediment accumulating behind them (Fig 3.5).

Figure 3.5*. Waterfalls in the tributaries of Sungai Temburong (top photos). Large debris dam (bottom left) on Sungai Esu, and a single tree trunk changing the flows (bottom right) from Sungai Engkabang.*

Intense rainfall frequently occurs in the late afternoon, causing water levels to rise rapidly over a short time period. This creates a continuum of conditions across two distinct environments (Fig 3.6). During low flows, tropical streams are complex systems with a mix of flow biotopes (e.g. pools, riffles and cascades) and functional habitats (e.g. wood debris, leaf litter, cobbles and gravel). Conversely, during a flood event, streams become homogeneous, with rising water forming a uniform flood biotope. As the main river expands in size, discharge increases and hydraulic damming and 'backup' occurs in the tributaries, with the main river flowing up the tributaries, until reaching a waterfall or sufficiently steep cascade. The base flow of some of the rivers and streams of the Ulu Temburong National Park are very low, and during dry weather, their channels may be reduced to a string of shallow pools (Fig 3.6). However, after a storm event, it is not unusual for river levels to rise by 3.5 m within an 8-hour period, this phenomenon being more pronounced in the deeply cut streams with stream banks of up to 70 degrees in slope (Choy and Chin, 1994).

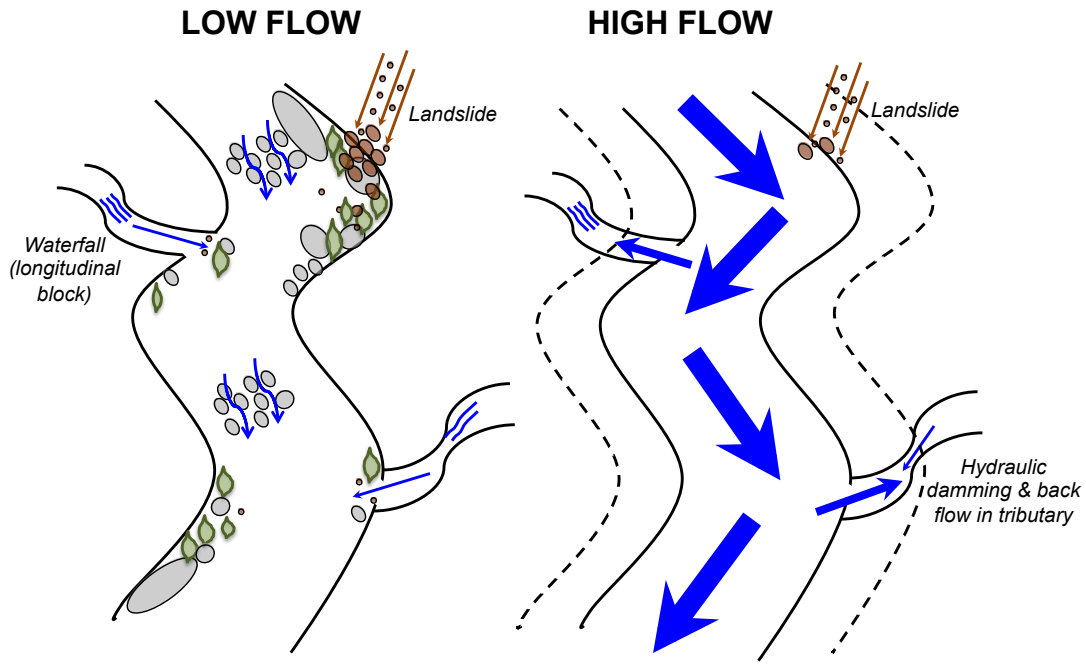


Figure 3.6. *Schematic illustrating how a tropical river channel changes from low to high flows, often daily. Low flow channel width is shown as thick dark black lines with the high flow channel width shown as dashed black lines. Blue arrows represent the water flow direction. Water can rise and fall within hours. Low flows support a mix of habitats and biotopes; high flows create a homogeneous channel and backs water up into the tributaries. Frequent landslides are a source of sediment and nutrients; waterfalls are a longitudinal block for animals and cause hydrologic disconnection from the main channel.*

Evidence of frequent storm events is shown in Figure 3.7. In April 2013, a debris dam measuring tens of meters in size was recorded at the confluence of Apan Tulan and Threelan (Fig 3.7A).The debris dam had been scoured out by the time fieldwork was recommenced one year later. Anecdotal evidence from the local boatmen suggests it was washed out during one large storm event in January 2014 (Fig 3.7B). In April 2016, hydraulic back flow occurred with Temburong flowing up Belalong (Fig 3.7C). The restricted river channels prevented Temburong from expanding laterally; therefore, once the water reached the confluence of Belalong, the water flowed upstream.

Figure 3.7. *Evidence of the frequent powerful flows on Sungai Temburong and Sungai Belalong (A) A large debris dam on Sungai Temburong in April 2013, with a (circled) man for scale. (B) Photo taken in the same place but one year later, after the debris dam had been washed away. (C) Water flowing up Sungai Belalong after a storm event on Sungai Temburong. The photo was taken at Kuala Belalong Field Study Centre (KBFSC), which is approximately 300 m from the confluence. Video shown here:* https://goo.gl/P1Z1i7*. Photo credits: (A) and (B) were taken by the author, and (C) was taken by Teddy Chua (KBFSC manager).*

In the smaller streams, the dense forest canopy prevents light from penetrating down to the channel. Cranbrook and Edwards (1994) found that often only 2–15% of visible light reaches the water surface. Despite this, there is diverse flora, and in the fast flowing sections,

algae is attached to submerged rocks, roots and plant stems. Bacteria and fungi are also important, with the later growing on submerged dead leaves (Cranbrook and Edwards, 1994). There are no aquatic macrophytes, but the banks and boulders do harbour rheophytes (Choy and Chin, 1994). The pH of the streams and rivers in Belalong is normally neutral, and the dissolved oxygen in the fast flowing areas usually exceeds 90%; however, in the lowland areas with high temperatures, fully saturated waters may contain only a few milligrams of oxygen per litre (Cranbrook and Edwards, 1994). As a consequence of leaching from soil and decaying organic matter, the sulphate content is usually high while levels of calcium and magnesium are usually low; this impacts biota such as molluscs and crustaceans. Phosphate is very scarce, as the forest is a closed system, with nitrogen levels being slightly higher and predominantly composed of ammonium and organic nitrogen (Cranbrook and Edwards, 1994). In a pilot study, both nitrate (<0.55 mg l^{-1}) and phosphate (<0.08 mg l^{-1}) were found to be below the level of detection. Water quality can have a substantial impact on benthic communities and ecosystem functions (Giller and Malmqvist, 1998; Everaert *et al.*, 2014); therefore, the high water quality in the study catchments and any variation in ecosystem structure and function must be assumed to be attributable to natural factors.

3.5.2 Background community structure

Choy and Chin (1994) recorded 44 species of fish belonging to 10 families and 30 genera from the headwater streams of Belalong and Temburong; 52% of these species are endemic to Borneo with the dominant fish families being Balitoridae and Cyprinidae. *Gastromyzon* (sucker fish), which are endemic to Borneo, are dorsoventrally flattened with enlarged pelvic fins that create adhesive pads; enabling them to cling onto rocks in fast flows (Sheldon, 2011; Fig 3.8). These grazing fish eat cyanobacteria, diatoms and filamentous algae. Sheldon (2011) found that visitation rates to algae-covered rocks were nearly four times higher relative to scrubbed ones.

There appears to be ecological zonation of fish and shrimp in the streams of Ulu Temburong National Park. Waterfalls are barriers to upstream colonization of fish, permitting shrimp and crabs to increase in density upstream. Cranbrook and Edwards (1994) found that 73% of fish species were found downstream of waterfalls. A mix of freshwater shrimp species from three genera occur in Ulu Temburong National Park (Wowor & Choy, 2004; Fig 3.8). The most abundant are *Macrobrachium* (Decapoda: Palaemonidae), which are

omnivorous but primarily predaceous (Wowor, *personal communication*). The other genera of shrimp include *Caridina* (Decapoda: Atyidae), which are normally present in the headwater streams, and *Atyopsis* (Decapoda: Atyidae), which are found in streams with strong currents (Wowor & Choy, 2001).

Figure 3.8*. Gastromyzon in in a pool in Sungai Baki (top left) and grazing scars in contrast to the fine layer of sediment that covers most of the rock (top right). Shrimp in Sungai Belalong (bottom left) and a flattened Mayfly Heptageniidae (bottom right). Images taken by the author.*

During the Rainforest Project (1991–1992), there were no quantitative studies conducted on the aquatic macroinvertebrate populations, and since this expedition, there have been few published studies that have focused on specific orders, with the limited research focused on Odonata, Plecoptera and Hemiptera (e.g. Zettle and Lane, 2010; Stark and Sheldon, 2009; Orr, 2001). The only published study that assessed the biogeography of macroinvertebrates in the Ulu Temburong National Park focused on Zygoptera (Orr, 2001). Meanwhile, there have been no published studies on macroinvertebrate community structure in the streams of Ulu Temburong. Due to the lack of identification keys for this thesis I have

been identifying macroinvertebrates down to the lowest taxonomic level then collaborating with taxonomists and research groups around the world (including the Natural History Museums in London, Singapore, Vienna and Lausanne,) to confirm my identifications and contribute to global taxa records. When the macroinvertebrates have not yet been described I created morphotypes.

3.6 Summary

The fieldwork presented in this thesis was conducted over three years. The first fieldwork trip was in July 2012 to Bukit Pagon, Brunei's highest mountain (Chapter 4), while the second (April to May 2013) and third (May to July 2014) research trips were to streams near to KBFSC. Chapter 4 describes a survey of macroinvertebrate communities inhabiting two streams conducted as part of the research expedition organized by the International Consortium of Universities for the Study of Biodiversity and the Environment and the Universiti Brunei Darussalam to Bukit Pagon. Chapter 5 gives a broad introduction to the macroinvertebrate communities of Temburong and Belalong, the largest rivers in Ulu Temburong National Park, and Chapter 6 is an investigation of the effect of Temburong and Belalong on their tributaries. Chapters 7 and 8 are focused on four tributaries, including Esu (a tributary of Belalong), Apan Threelan, Baki and Lower Apan (all tributaries of Temburong; Fig 3.9). These tributaries were generally steep with a mix of biotopes, including numerous waterfalls. Chapter 7 characterizes the effects of biotopes on macroinvertebrate community structure, and Chapter 8 examines aquatic communities, litter decomposition and periphyton growth rates for above- and below-waterfall pools. Chapter 9 presents a stable isotope analysis and gut contents analysis to investigate the trophic structure of dominant macroinvertebrates living among waterfalls.

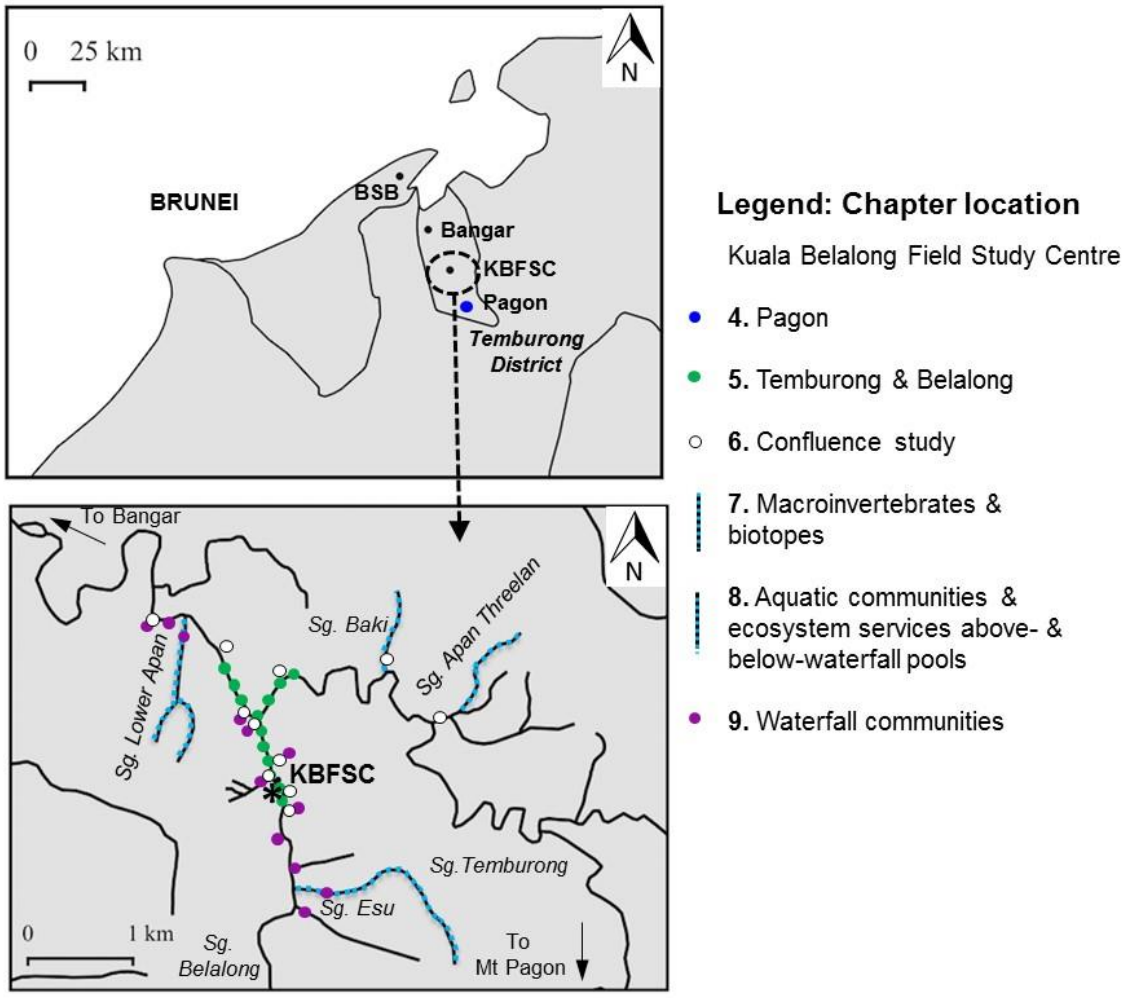


Figure 3.9. *Brunei, with the Temburong district shown on its eastern side (top map). The southern part of the Temburong district is Ulu Temburong National Park, Kuala Belalong Field Study Centre (KBFSC) and Pagon base camp are highlighted. All the study sites corresponding to Chapters 4–9 are highlighted in the bottom map.*

.

Chapter 4: Eco-Hydromorphic Classification for Understanding Tropical Stream Macroinvertebrate Biodiversity[1]

4.1 Introduction

It is widely recognised that interactions between hydrology, sediment dynamics and river morphology controls the size, pattern and habitat structure of river channels (Brierley *et al.,* 2013; Villeneuve *et al.,* 2015). These linkages are responsible for making the river channel highly diverse, due to the different and specific composition of the patches, which support diverse macroinvertebrate communities (*i.e.* eco-hydromorphic complexity; *sensu* Townsend 1996, Hannah *et al.,* 2004). Consequently, eco-hydromorphology plays a crucial role in comprehending the spatial and temporal diversity of river habitats. Classification and mapping of eco-hydraulic patch complexity provides a robust technique for exploring how stream habitats are formed and maintained (Poff and Ward 1989; Hart and Finelli 1999; Bunn and Arthington 2002). In temperate streams, eco-hydrogeomorphology has become a cornerstone for investigating ecological patterns, with flow widely recognised as the master variable which regulates community structure of both vertebrates and invertebrates (Poff and Ward 1989; Hart and Finelli 1999; Stubbington *et al.,* 2009). In contrast, few studies have examined eco-hydromorphic complexity in tropical streams (however see: Ramírez and Pringle 1998; Boyero 2003; Boulton *et al.,*. 2008; Principle 2008; Md Rawi *et al.,* 2014).

Classification concepts organise information, providing a systematic and repeatable method to view the eco-hydrogeomorphic complexity of stream ecosystems (Brierley and Fryirs 2008). One approach to classifying stream habitats is to describe a set of biotopes (Jowett 1993; Wadeson 1995; Padmore 1998; Newson and Newson 2000; Clifford *et al.,* 2006). A biotope is defined as a "habitat assemblage with a characteristic range of temporally variable hydraulic and substrate characteristics which can be associated with the morphological units" (Wadeson 1995, p7). As such, biotopes are a useful classification tool

[1] This chapter is published in the Journal of Zoological Studies

as they can be observed as river surface flow features (flow/hydraulic biotopes; Table 2.1) such as riffles and pools, which reflect combinations of sediment, depth and velocity associated with the organisation of the river bed (*i.e.,* physical biotope; Harvey *et al.,* 2008). In the tropics, biotopes have been used as a sampling framework for a few studies (Furtado 1969; Quentin 1973; Dudgeon, 1994), while some studies have examined pools and riffles to assess longitudinal assemblage structure in tropical rivers (*e.g.*, Furtado 1969; Bishop 1973; Rundle *et al.,*. 1993; Greathouse and Pringle 2006). However, there has been little research on how the structure, composition and pattern of biotopes affect macroinvertebrate biodiversity (Ramírez and Pringle 1998; Boyero 2003; Cheshire *et al.,* 2005; Md Rawi *et al.,* 2014).

Along with biodiversity, macroinvertebrate size structure within biotopes is important because body size influences many ecological processes (Schoener 1986). For example, macroinvertebrate size structure affects the structure and dynamics at the community level as it influences potential resource use and impacts predation rates (Schmid *et al.,* 2000; Woodward and Warren 2007). Differences in macroinvertebrate size structure have been found in runs and riffles in Costa Rica and these differences were suggested to indicate different ecological functions at the biotope scale (Principe 2008). Despite there being numerous studies on macroinvertebrate size structure in temperate streams (*e.g.*, Lafferty and Kuris 2002; Woodward *et al.,* 2005; White *et al.,* 2007; Dial *et al.,* 2008) there are few tropical streams studies that have focused on macroinvertebrates community size structure in different biotopes (however see Principe 2008).

Quantification of habitat use assists in the prediction of macroinvertebrate responses to changes in habitat availability (Hawkins *et al.,* 1993). Extrapolation of ecosystem models based on temperate streams may not apply to tropical systems and management techniques used in temperate areas may not work or potentially damage tropical systems (Boulton *et al.,* 2008). Therefore, the aim of this chapter is to investigate patterns of macroinvertebrate biodiversity and size structure using the Biotope concept.

4.2 Methods

4.2.1 Study Sites

This project was part of an expedition to Bukit Pagon (1850m), Brunei's highest mountain. The main goal of the expedition was to conduct a systematic study of the environment and biodiversity of high altitude primary rainforest in Brunei (Fig 4.1A). Prior

to this expedition, no previous scientific studies had been conducted. Base camp was situated on the slopes of Bukit Pagon (4º33'614"N, 115º26'153"E), set up at an elevation of 862 m.a.s.l and 30 km away from the nearest road and only accessible by helicopter (Fig 4.1A).

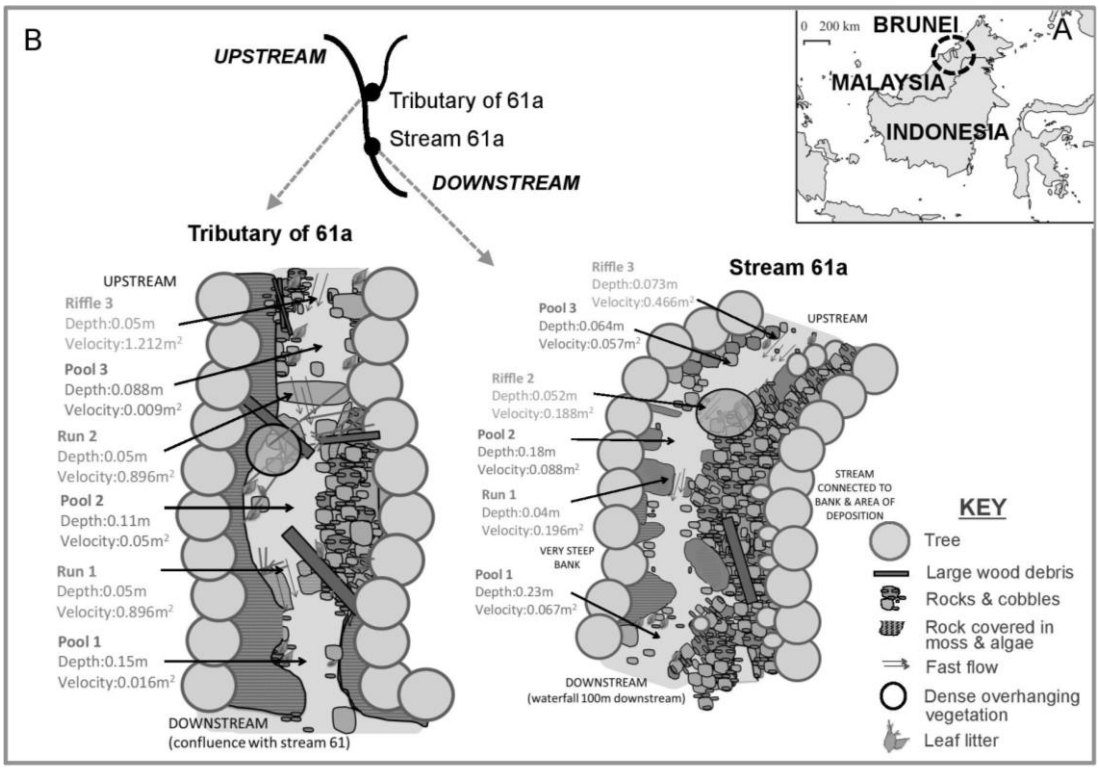


Figure 4.1. *Map and schematic of study sites A) Map of Borneo Island in S.E Asia with Brunei highlighted. B) Plan view schematic of the two study sites, tributary of 61a (left) and stream 61a (right).*

The dominant vegetation type was sub-montane heath forest (Ahmad Sah *et al.,* 2006). The study reaches were less than 10% in gradient with no waterfalls present. Two streams close to the base camp were the focus of this study (stream 61a and tributary of 61a; Fig 4.1B). In each stream, three repeating fast/slow habitat units being approximately 50-meters in length defined the reach. Sampling took place from 4-6th July 2012. Stream 61a was a larger stream with its tributary entering it upstream of the designated study reach.

4.2.2 Field Methods

The fast/slow habitat units in each reach were mapped as biotopes (pools, riffles and runs) by observing river surface features (Newson and Newson 2000, Parasiewicz 2007; Table 2.1). For each biotope a formal cross-section of velocity and depth was taken every 0.5m following common methods described by Gorden *et al* (2004). In addition both wetted

width (defined by the area of stream channel filled with water) and bank-full width (defined by the width of the river during high discharge) was measured. Channel dimensions were measured with surveying tapes and metre sticks. Stream velocity was measured at 60 % depth as suggested by Gordon *et al* (2004), using an electromagnetic flowmeter (Valeport® model 801; Valeport Ltd., Totnes, UK). Discharge (Q) was calculated for each stream using the following equation:

$$\text{Discharge (Q) } m^3 s^{-1} = \text{Velocity (V) } ms^{-1} \text{ x Cross sectional Area (A) } m^2$$

For the whole biotope unit, the benthic substrate was assessed visually using a collapsed version of the Wentworth scale categorising the percent coverage of gravel, cobble, boulder and bedrock (Gorden *et al.,* 2004). The presence or absences of wood debris, leaf litter, and moss in all biotopes were also recorded. Benthic macroinvertebrates were sampled in each biotope using a Surber sample ($0.10m^2$; 1mm mesh) with three replicates taken randomly in each biotope. Due to low densities of macroinvertebrates, three samples in each biotope were composited to create one intact sample.

4.2.3 Laboratory Methods

Macroinvertebrate samples were processed in the field in 70% Ethanol. Once exported to the UK, collected individuals were identified to the lowest practical taxonomic level, enumerated and measured to the 0.5 mm under a microscope. Identifications were conducted mainly using taxonomic keys from Dudgeon (1999) and Yule and Yong (2004b). However, given the paucity of macroinvertebrate taxonomic knowledge in Borneo, open source identification methods were used. Specifically, taxa were photographed, highlighting the distinguishing morphologies and uploaded onto the Flickr website (flickr.com/photos/tropical-streams/sets/) where interested experts could comment on specimens. This method was a useful tool to confirm identifications via input from appropriate research groups and taxonomists from around the world. Most taxa were identified to genera or morphotyped to similar level. Some taxa, particularly specimens in the Orders Coleoptera and Diptera, which are significantly under researched in northern Borneo, could only be identified to the family level (Manfred, personal communication, 2014; Yule 2004).

Body lengths (not including appendages and setae) of sampled individuals were measured to the nearest 0.5 mm to estimate taxa-specific ash free dry mass (AFDM) using length- mass regressions (Benke *et al.,* 1999; Sabo *et al.,* 2002; McNeely *et al.,* 2007). In cases where no taxa-specific equations exist, estimates were made using equations from taxa with similar body shape following Ramírez and Pringle (1998). Where only dry mass (DM) estimates are available, values were converted to AFDM following Waters (1977). Individual body lengths were also used to investigate patterns of macroinvertebrate size structure between the biotopes. Macroinvertebrates were placed into 6 size classes: I= >0-2mm, II= 2.1-4mm, III=4.1-6mm, IV= 6.1- 8mm, V= 8.1-10mm, VI= 10.1-20mm.

4.2.3 Data Analysis

To assess macroinvertebrate biodiversity, richness, density and biomass were quantified in all the fast and slow biotopes in each study streams. T-tests were used in this paper to compare the physical variables (width, depth and velocity) and biodiversity (mean density and biomass per unit area, and richness) between the tributaries and fast/slow habitats. The statistical analysis of T tests was chosen based on the distribution of the data (Thomas *et al.,* 2013). Physical variable structure was examined within the biotopes using a hierarchical cluster analysis (dendrogram) carried out based on Gower Coefficient, which can handle nominal, ordinal, and asymmetric binary data (Gower 1971). Macroinvertebrate assemblage structure was also examined within the biotopes using a dendrogram but based on Bray Curtis Coefficient, a popular similarity index for ecological data (Borcard *et al.,* 2012). Bray-Curtis similarity matrices calculated the compositional dissimilarity of sites based on the abundance and biomass of taxa at each site. Multi-dimensional scaling (MDS) analysis with the Bray-Curtis dissimilarity was used to test the robustness of groups defined by the dendrogram. A BIO-ENV (Clarke and Warwick, 2001) analysis was carried out to investigate which environmental variables best correlate with macroinvertebrate community structure. Similarity percentage analysis (SIMPER) was used to identify taxa, which contributed most to the average dissimilarity between biotopes. Kolmogorov-Smirnov test was used to assess any differences in the taxa size structure among the biotopes by comparing the general shape of distributions (Thomas *et al.,* 2013). Statistical analysis was carried out in the statistical computing environment R (R Core Team 2013) using the Vegan, Cluster, and Labdsv packages.

4.3 Results

4.3.1 Site Description

In both study reaches, slow flow biotopes (pools) were interspersed with fast flow biotopes (riffles and runs; Fig 4.1B). The tributary had three pools, two runs and one riffle while the stream 61a study reach had three pools, one run and two riffles. Overall, both study reaches had similar average wetted widths of all the biotopes (tributary - 2.1 m; stream 61a - 3.2 m; Table 4.1). However, average bankfull width of all the biotopes was twice as wide for stream 61a than the tributary (stream 61a - 7.0 m vs. tributary - 3.8 m). Average biotope depths between the two study reaches were similar (tributary - 0.09 m vs. stream 61a - 0.10 m). Discharge was slightly higher at stream 61a (0.18 m^{-3} s^{-1}) compared to the tributary (0.12 m^{-3} s^{-1}). However, average velocities were faster in the tributary (0.54 m s^{-1}) compared to stream 61a (0.21 m s^{-1}).

Table 4.1. *Channel characteristics and physical conditions of the biotopes at stream 61a and at the tributary. Type C = cobbles, G = gravel, B = boulders and BR = bed rock*

	Bankfull width (max) m	Wetted width (max) m	Depth (max/avg) m	Velocity (average) m s^{-1}	Dominant substrate type			
Slow flows					**C**	**G**	**B**	**BR**
S.Pool 1	6.20	3.47	0.37/ 0.23	-0.07	70	20	10	0
S.Pool 2	6.40	2.90	0.38/ 0.18	-0.09	60	10	0	30
S.Pool 3	6.70	2.65	0.13/ 0.06	-0.06	80	10	10	0
T.Pool 1	2.50	2.50	0.22/ 0.15	-0.02	50	40	0	10
T.Pool 2	4.70	2.50	0.21/ 0.10	-0.05	20	30	0	50
T.Pool 3	3.16	2.60	0.16/ 0.11	-0.01	80	15	5	0
Average	4.94	2.77	0.138	-0.05	60	21	4	15
Fast flows								
S.Run 1	6.10	1.02	0.05/ 0.04	0.20	5	0	0	95
S.Riffle 2	9.40	4.27	0.07/ 0.04	0.79	30	20	50	0
S.Riffle 3	7.30	2.47	0.15/ 0.05	0.47	85	15	0	0
T.Run 1	3.90	0.87	0.07/ 0.04	1.20	5	5	5	85
T.Run 2	3.57	2.04	0.05/ 0.05	0.90	0	5	0	95
T.Riffle 3	4.70	1.90	0.04/ 0.06	1.21	65	15	20	0
Average	5.83	2.10	0.047	0.79	32	10	12	46

Slow flow biotope wetted widths were approximately 1 metre less in the tributary compared with the stream, while bankfull widths ranged from 6.70 m (stream 61a-pool 3) to 2.50 m (tributary- pool 1) as shown in Table 4.1. Fast flow biotopes, including riffles and runs, had wetted widths ranging from 4.27 m (stream 61a-riffle 2) to 1.90 m (tributary-riffle 3), while bankfull widths ranged from 9.40 m (stream 61a-riffle 2) to 3.57 m (tributary-run

2). As expected, slow flow biotopes (0.06 m to 0.23 m) were deeper than fast flow biotopes (0.04 m to 0.06 m). Velocities in slow flow biotopes were mainly negative, while velocities in fast flow biotopes varied from 0.20 m s^{-1} (stream 61a-run 1) to 1.21 m s^{-1} (tributary-riffle 3). These physical measurements are summarised in Table 4.1.

The tributary was constrained by the riparian bedrock, resulting in narrower bankfull widths and a smaller canopy gap with less sunlight reaching the channel (Fig 4.1B). This constrained reach also contained more wood debris (large and small), leaf litter packs and the associated build-up of fine sediments and organic debris. In contrast, the wider reach at stream 61a had more sunlight reaching the channel that promoted filamentous algae and moss in fast flow biotopes. Further, woody debris accumulated in one fast flow biotope (stream 61a-riffle 2; Fig 4.1B).

The dominant substrate types in both study reaches were similar among slow flow biotopes with a mix of cobbles, gravel and boulders (Table 4.1). However, tributary-pool 2 and stream 61a-pool 2 also had large amounts of bedrock. Substrate at the fast flowing biotopes varied with runs being primarily bedrock (> 80% coverage) and riffles being comprised of a mix of cobbles, gravel and boulders (Table 4.1).

Dendrogram of the physical variables among biotope habitats (*see* Table 4.1) shows a 0.7 dissimilarity between the runs compared to other biotopes (Fig 4.2A). Variation in substrate type separated runs from the other habitats (i.e., runs being mainly composed of bedrock). As such, some pools and riffles clustered together due to similarities in their physical conditions. However, tributary-pool 2 shows a 0.3 dissimilarity from all other pools and riffles and is explained by the large amount of bedrock present (*e.g.*, 50%).

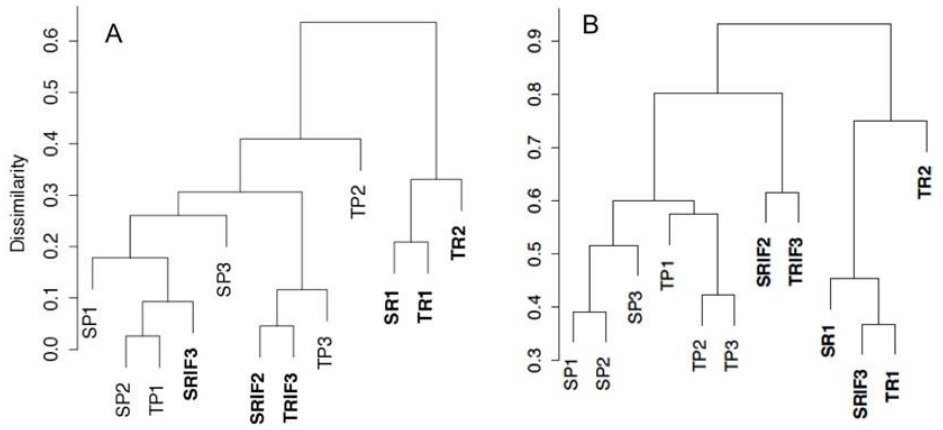


Figure 4.2. *Dendrogram of A) biotope physical conditions and B) biotope taxa abundance. The first letters represents the study site: S= Stream 61a, T= tributary. The second letter and the number represents the biotope sampling unit: R= run, RIF= riffle, P=pool. Letters in bold represent the fast flowing biotopes.*

4.3.2 Biotope Taxa Distribution

The two reaches were similar in terms of their overall macroinvertebrates biodiversity (Fig 4.3 and see Appendices Chapter 4a&b). Taxon richness was the same at both sites (p= 1). Average density was not significantly different (p= 0.09), with Stream 61a having 85 ± 18 individuals per m^2 compared to 60 ± 8 individuals per m^2 in the tributary. Average biomass (AFDM) was approximately 80% less in the tributary when compared to stream 61a (tributary: 38 ± 10 mg m^{-2}; stream 61a: 167 ± 102 mg m^{-2}), but no statistical differences were found between the two reaches (p= 0.25). The high average biomass at stream 61a is explained by the large Nepidae (Cercotmetus) found at SP3, without the water scorpion average biomass at stream 61a would be ~ 68 mg m^{-2}.

Differences in macroinvertebrate biodiversity were found among habitat types in the two study reaches (Fig 4.3). Slow flowing biotopes had significantly higher average richness (11) compared to the faster flowing biotopes (8) (p= 0.05*) and riffles had 38% more taxa than runs (11 versus 5). Macroinvertebrate densities were similar (p=0.86) among fast flow biotopes (average all fast habitats - 71 ± 18 individuals per m^2; runs - 75 ± 31 individuals per m^2; riffles - 66 ± 25 individuals per m^2) and slow biotopes (74 ± 8 individuals per m^2; Fig 4.3). On average, macroinvertebrate biomass was lower in fast flowing biotopes (55 ± 21 mg AFDM m^{-2}) than slow flow biotopes (149 ± 104 mg AFDM m^2). However, there was no statistical difference between the fast and slow biotopes (p= 0.45). Pool biomass was 50% higher than the runs and 24% higher than the riffles.

Dendrogram of taxa abundances showed a 0.9 dissimilarity between the 2 run biotopes (SR1 and TR1) and 1 riffle (SRIF3) with the rest of the biotopes. It also showed a 0.8 dissimilarity between the other 2 riffles (SRIF2 and TRIF3) and all the pool biotopes (Fig 4.2B). MDS ordination of taxon abundances (Fig 4.4) showed distinct community structure among the biotopes (stress = 0.01; Clarke and Warwick, 2001).

The ordination identified three main groups of taxa, associated with the 3 biotopes: pools, runs, and riffles. Taxa found in pools were associated with wider wetted widths, gravel, cobbles, and deeper depths. The taxa found in runs were associated with moss, higher percentage of bedrock, and faster velocities. While the taxa found in riffles were associated with wider bankful widths, wood debris, leaf litter, and faster velocities. BIO-ENV shows the best single environmental factor that correlates to the taxa include cobble substrate (rank correlation=0.65), whilst the best 3 environmental factors combined were velocity, cobbles and gravel (rank correlation=0.70). As expected, pools had a strong association with depth

and a negative association with velocity, whilst riffles were least associated with depth and most related to high velocities (Fig 4.4). Cobbles, gravel, and leaf litter were associated with both riffles and pools. Bedrock and moss were most strongly associated with runs.

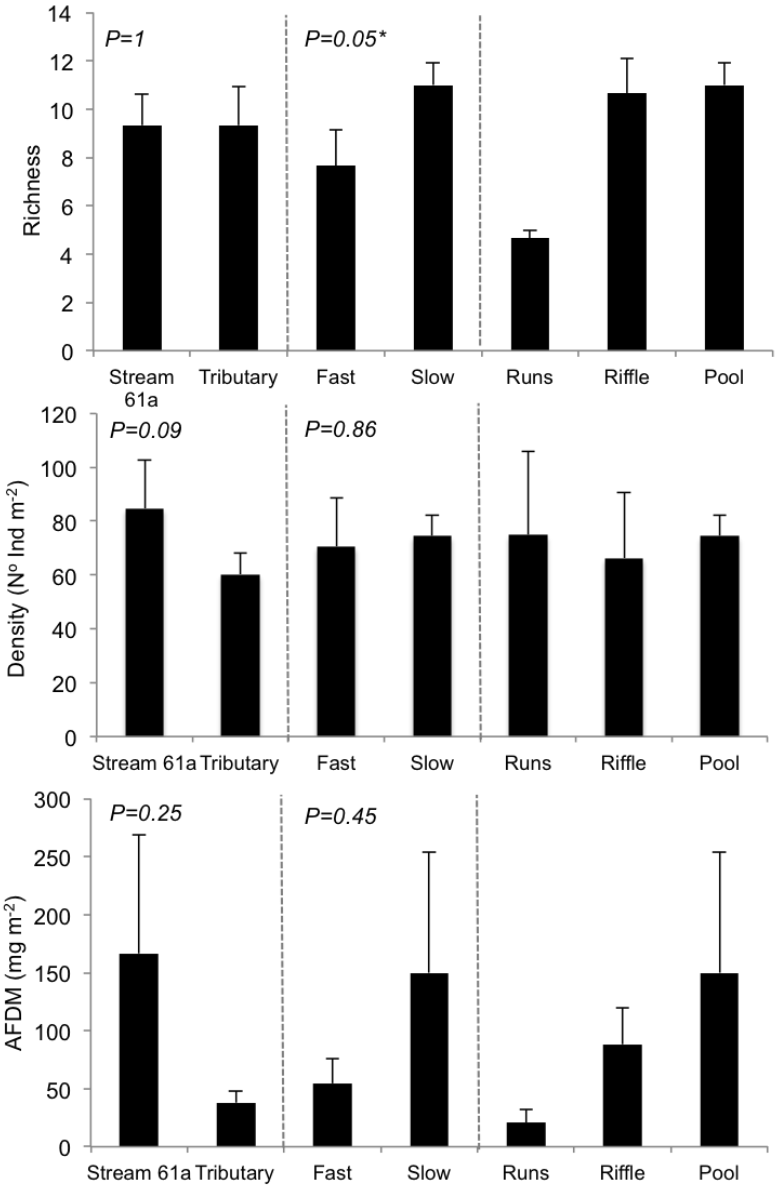


Figure 4.3. *Bar plots with richness, density, and biomass of macroinvertebrates at the two study sites. Includes bar plots of stream 61a and tributary, the fast and slow flowing habitats, and the three biotopes (runs, riffles and pools). Error bars represent standard deviation (Stream 61a n= 6, tributary n=6, both fast and slow flowing habitats n=6, runs n=3, riffle n=3 and pool n=6). Significant differences between the fast and slow sites with richness (p=0.05*).*

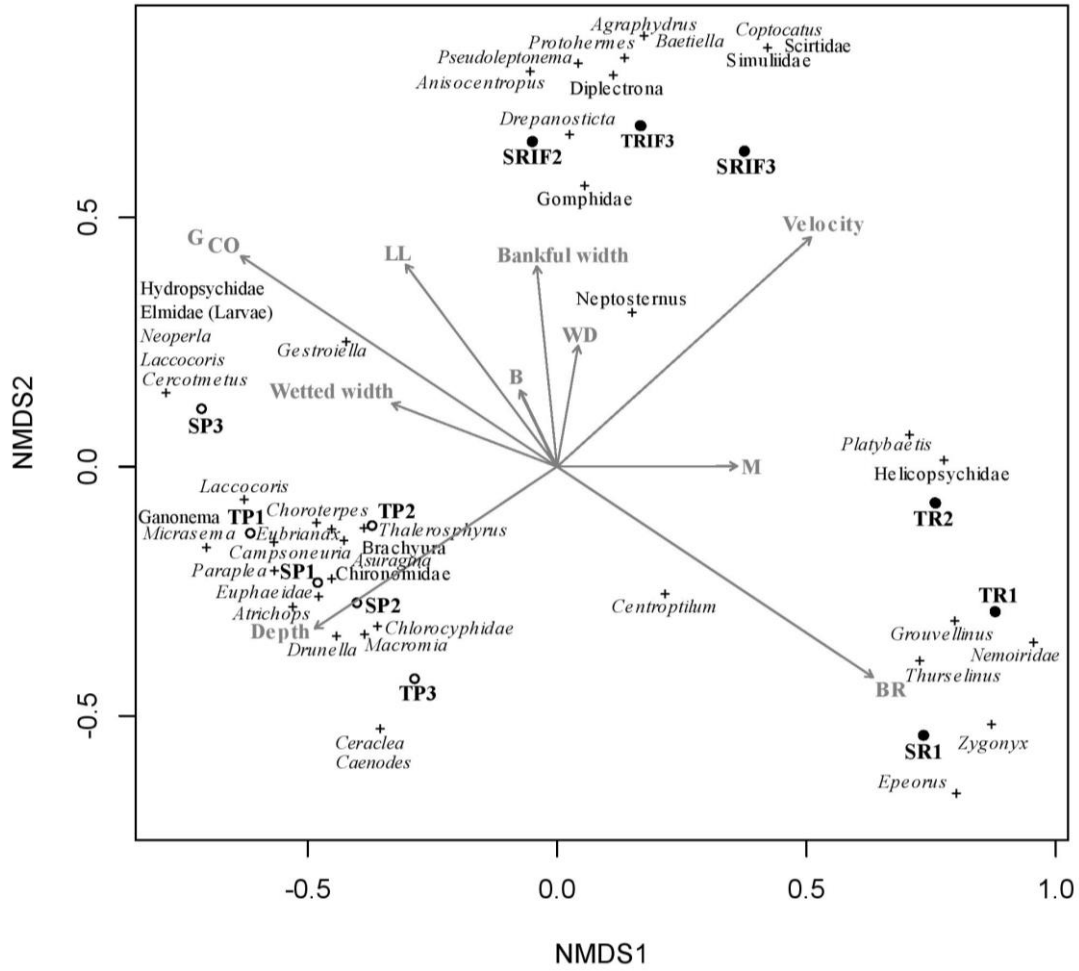


Figure 4.4. *MDS ordination with a BIO-ENV that shows the environmental variables that best correlate with macroinvertebrate community structure. The biotope names have been abbreviated with the first letter representing the study site: S= Stream 61a, T= tributary. The second letters represent the biotopes: R= run, RIF= riffle, P=pool. The environmental data in grey with G=gravel, CO= cobbles, LL= leaf litter, B= boulder, BR= bedrock and WD= wood debris, M=moss. Stress: 0.01.*

SIMPER analysis showed average similarity between taxa was highest in the pools at 44% and lowest in the riffles at 27% (Table 4.2). In pools, *Thalerosphyrus* species (Ephemeroptera: Heptageniidae) contributed about 30% to the total similarity, followed by *Eubrianax* species (Coleoptera: Psephenidae) at nearly 20%. In the riffles average similarity was only 27%, with Gomphidae contributing 40% to the total. In the runs, where average similarity was 37%, *Grouvellinus* species (Coleoptera*:* Elmidae) contributed 78% to the total.

Table 4.2. *SIMPER analysis of the top taxa contributing to the observed similarities between the biotopes. Summary results show the percentage contribution and the cumulative contribution of contributing taxa to each biotope with percentages.*

	Percentage contribution (%)	**Cumulative contribution (%)**
Pool: Average similarity: 44.09		
Thalerosphyrus	29.6	29.6
Eubrianax	19.6	49.2
Choroterpes	17.2	66.4
Campsoneuria	9.5	75.9
Chironomidae	5.8	81.7
Run: Average similarity: 37.60		
Grouvellinus	78.3	78.3
Platybaetis	6.1	84.4
Helicopsychidae	6.1	90.5
Riffle: Average similarity: 26.76		
Gomphidae	40.0	40.0
Protohermes	20.0	60.0
Campsoneuria	9.6	69.6
Macronematini	9.6	79.2
Thalerosphyrus	5.5	84.7
Diplectrona	5.5	90.2

4.3.3 Macroinvertebrate Size Structure in the Biotopes

Average taxon lengths in the two reaches showed a similar pattern, with most taxa occurring in size class II (Fig 4.5). There was a significant difference (p=0.04) between the taxon lengths found in fast and slow flow biotopes. A larger proportion of taxa present in fast flow biotopes were found in the smallest size class. Differences in taxa length between the biotopes were also evident; significant difference between pools and runs (p=0.01) and between riffles and runs (p=0.01). Taxa present in runs were only found in the smallest three size classes, with the highest number of taxa in size class I. Pools and riffles had a similar distribution among size classes (p=0.10); the only difference being that taxa in riffles had a higher average number of individuals in size range I and size range VI.

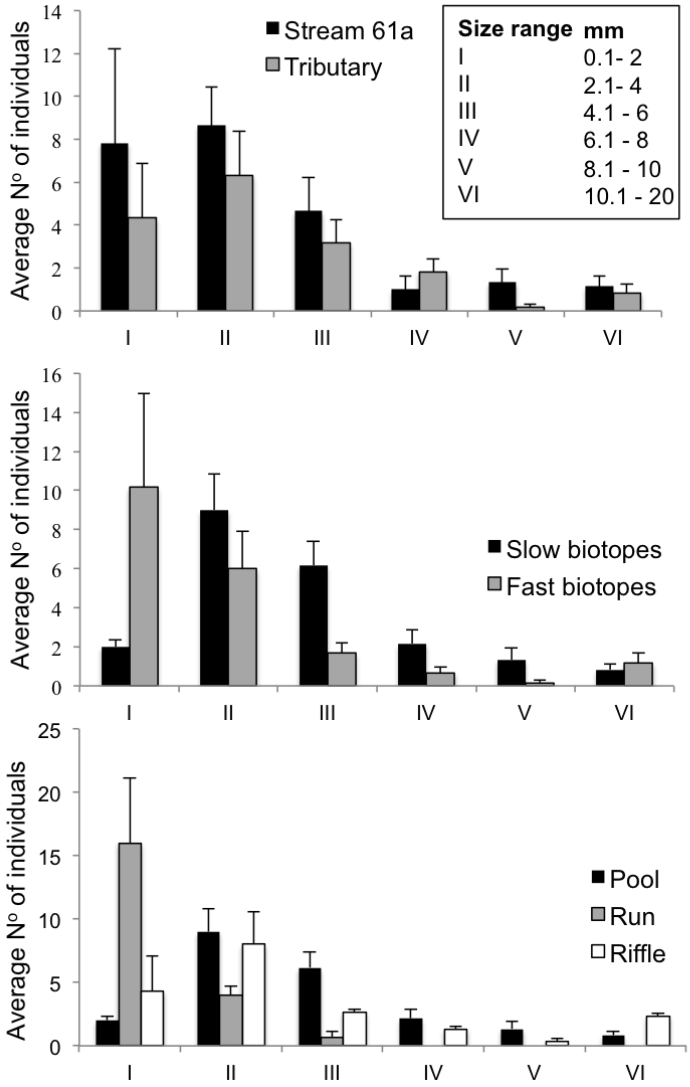


Figure 4.5. *Length- frequency distributions of macroinvertebrates among stream 61a and its tributary, fast and slow biotopes, and among the three biotopes (runs, riffles and pools). Error bars represent standard deviation (Stream 61a n= 6, tributary n=6, both fast and slow flowing habitats n=6, runs n=3, riffle n=3 and pool n=6). Significant differences found between the fast and slow sites (p= 0.04*), pool and runs (p=0.01) and riffles and runs (p=0.01) using Kolmogorov-Smirnov test.*

4.4 Discussion

This project used the eco-hydrogeomorphic concept of biotope as a framework to investigate the biological and physical relationships of two streams draining Bukit Pagon. The sites were less than 100m apart, under the same environmental conditions of discharge regime, geology and climate, and as a result had very similar physical conditions. These similarities allowed for a critical examination of fast and slow flow biotope and the associated macroinvertebrate biodiversity.

At both study streams macroinvertebrate diversity was lower compared to a similar system in Malaysia (204 taxa; Bishop 1973). However, there are examples of tropical lotic ecosystems with values of richness close to those measures in my system, for example 53 taxa were found in Sabalo stream, Costa Rica (Ramírez and Pringle 1998) and 52 taxa in the Rio Camuri Grande, Venezuela (Cressa 1998). However, comparing richness among all of these datasets should come with caution due to the incomplete knowledge of tropical macroinvertebrates and different sampling techniques (Jacobsen *et al.,* 2008). For example, I used a large mesh size (i.e., 1 mm), which may have resulted in a loss of small taxa. However, these "missing taxa" would likely be chironomid taxa (Insecta: Diptera: Chironomidae) taxa, which are underrepresented in many stream ecology studies (Ferrington 2008; Armitage *et al.,* 2012).

Macroinvertebrate density and biomass were also low at the two study reaches, with average density of 72 individuals per m^2 and average biomass of 102 mg AFDM m^{-2}. Graça *et al* (2015) found an average of 150 to 300 individuals per m^2 in eight headwater streams in Brazil, whilst Boyero and Bailey (2001) study on riffle habitats in Panama found an average density of 905 individuals per m^2. The low densities in my study reaches may be explained by abiotic factors, with disturbance caused by flooding spates having a strong influence on macroinvertebrate diversity (Power *et al.,* 1988; Resh *et al.,* 1988; Death 2002). The study streams draining Bukit Pagon are similar to many tropical headwaters, characterised by frequent flashy storm hydrographs and spates which tend to lead to significant scouring of individuals that lack refugia from elevated flows (Boulton *et al.,* 2008). Another reason for low densities in the tropics could be due to the higher levels of predation by both macroinvertebrates and fish (Fox 1977).

Padmore (1998) highlighted that biotopes are not static units with most turning into deep runs under flood conditions. In Bruneian streams, this continuum of conditions can occur frequently, with daily storms during most months, creating two distinct stream environments. During low flows, Bruneian streams are complex systems with a mix of flow biotopes (pools, riffles, waterfalls) and other habitats (wood debris, leaf litter, cobbles, gravel). Conversely during flood events, these streams become homogeneous forming one flood biotope. As many tropical systems have this natural and consistent flow disturbance, other types of habitats become vital areas of refuge. This is especially true of habitats that can withstand scour, such as bedrock. Bank irregularities and flow obstructions such as large

rocks are also important in creating heterogeneous hydraulic conditions throughout a range of flow stages, thereby providing another refuge (Harvey and Clifford 2009).

Interestingly the two Bray Curtis dendrograms, one with physical (Fig 4.2A) and the other with the taxa data (Fig 4.2B), show taxa fitting the biotopes more strongly than the physical data. The flashy flows in tropical streams create a very dynamic geomorphic template, making it difficult to find patterns with the physical data during a one off survey. In comparison, this study suggests that the taxa reflect the biotopes more accurately than single physical measurements. This is not surprising given that the communities often tell the 'longer story' (*e.g.*, macroinvertebrates being used as indicators for water quality; Giller and Malmqvist 1999).

Despite the broader pattern of macroinvertebrates reflecting the biotope framework, there is a hierarchy of factors that affect the distribution of each taxon, which would explain why few taxa were uniquely associated with certain biotopes. These findings are similar to results from temperate streams, which concluded that due to the range of habitats and life stages, along with synecological factors (such as competition and predation), it is difficult to find distinct macroinvertebrate communities within individual flow biotopes (Newson and Newson 2000). Macroinvertebrate communities may also be affected by the configuration and hydraulic properties of biotopes, with biotopes in the same class being highly variable depending on how they are formed and their position in the channel (Bisson *et al.,* 1982). Other habitat parameters (*i.e.*, leaf litter and moss) can be flow independent. As a result, these factors can be found in all flow biotopes influencing macroinvertebrate distribution and biodiversity regardless of the dominate flow types. In this study, the pools and riffles had more leaf litter and wood debris than the runs.

Runs had swift current velocity, but limited leaf litter and wood debris. Water flowing over the runs was smooth with little turbulence or spray resulting in low hydraulic heterogeneity. This could be a reason for the lowest biomass (AFDM) and richness at both study reaches (Fig 4.3). However, these conditions appear to be particularly good for *Grouvellinus* species (Coleoptera: Elmidae) based on the high densities in runs, especially at SR1. These elmids were very small, few were >1.5mm therefore not increasing overall biomass. Other habitat parameters appear to be important in the faster flowing biotopes. For example, *Grouvellinus* species and *Zygonyx* species (Odonata: Libellulidae) are known to cling to hard substrates covered by moss, to help them withstand fast flows (personal observations). Furtado's (1969) analysis of Odonates in a Malaysian stream found that

Zygonyx species managed to inhabit trailing plants and accumulated debris irrespective of water velocity. The morphology of *Zygonyx* species with spines covering the whole body aid attachment, enabling the animal to use moss as refuge from a range of flow velocities. It is clear that moss and other habitats (*i.e.*, leaf litter) can have strong influences on both body size and biomass in fast flowing biotopes. In support of this, Rackemann *et al* (2013) emphasised the need for further investigation into the role of moss in protecting insects and therefore maintaining higher diversity in another fast flow biotope - waterfalls.

Body size can influence an organism's ability to withstand fast flows. Results from this study show that taxa in pools are generally larger, and that communities were composed of smaller number of taxa, in fast flow biotopes. This is particularly evident in the runs, with communities dominated by elmids and simulids. For example, 75% of organisms present at run 1 (stream 61a) was attributed to the family Elmidae. Their small body size enables them to take refuge from fast flows within the crevasse of the bedrock, moss or algal-covered patches. In pools average size of taxa was slightly larger, animals such as chironomids and oligochaetes, were able to avoid high flows by burrowing into the fine sediments (Stubbington 2009). These refuges evidently provide protection for a wider range of body sizes than habitats available in runs. The increased range of macroinvertebrate body sizes found in the pools and riffles potentially reflects the diverse mix of habitats.

Collecting representative samples in any environment is difficult but it is especially problematic in the tropics because species diversity is high but many species are rare (Gotelli and Colwell, 2011; Chao *et al.,* 2014; Hsieh *et al.,* in press). In my study I found that with 6 replicates of the fast and slow biotopes species diversity is ~ 25 (Fig 4.6A). If I doubled my sampling effort to 12 replicates, species diversity is expected to increase to ~30-35 taxa (Fig 4.6A). However, if I eliminate the rare taxa (classed as taxa occurring only once), it shows that I have collected the common taxa (Fig 4.6B). In the tropics it is not uncommon for biodiversity surveys to miss taxa due to the high number of rare taxa, creating a slowly rising species accumulation curve (Gotelli and Colwell, 2011). In this study the main aim was to make comparisons of the biotopes during one time period, when comparing community structure between the biotopes this will be dictated mainly by the common taxa.

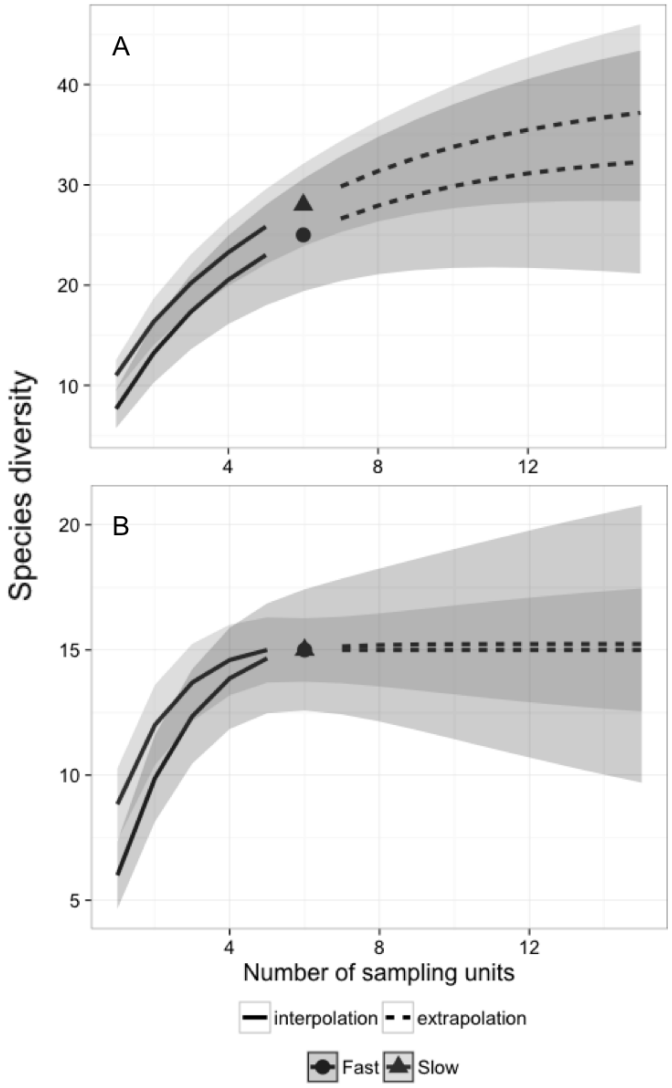


Figure 4.6. *Sample-size-based rarefaction and extrapolation for fast (runs and riffles) and slow (pools) flow biotopes. Graph (A) includes all sampled taxa and (B) includes the common taxa which is defined as any taxa that is found more than once.*

4.5 Conclusions

Streams are heterogeneous and extremely dynamic in nature. Biotopes are a logical place to start understanding this complexity as they can be easily observed as river surface flow features, which reflect hydraulic and sediment characteristics. This study has shown that biotopes are useful for examining macroinvertebrate biodiversity in streams, with richness and community structure reflecting conditions at the biotope scale rather than at the reach level. Cluster analysis also showed distinct community structure among the pools, runs, and riffles. Macroinvertebrate size structure among biotopes was distinct between fast and slow flows, with the smallest taxa being most abundant in runs. This study suggests that further research is required to understand the importance of a range of habitat parameters, which

are not directly related to flow velocities. These habitat parameters are important as refuge and allowing the colonisation of habitats that would otherwise be inhospitable during flood periods.

The timeframe to investigate and understand basic tropical stream ecology is put under pressure due to the strong influence of man on these ecosystems (Dudgeon *et al.,* 2006). Given environmental changes and associated loss of biodiversity, it is imperative to collect baseline data and understand the processes and roles of natural pristine tropical systems. This will enable understanding of the potential consequences of extinction and declining biodiversity (Dudgeon *et al.,* 2006; Corlett 2009).

Chapter 5: Macroinvertebrate Diversity in Pools of Sungai Temburong & Sungai Belalong: A Short Communication

5.1 Introduction

Current understanding of macroinvertebrate biodiversity of tropical rivers (author's note, rivers for this dissertation are defined as non-wadeable systems) is relatively limited compared to temperate regions (Dudgeon, 2008). Further, knowledge of tropical rivers in South East Asia are even more scant compared to other tropical regions such as northern Australia and the Neotropics (Mantel *et al,* 2004; Ho and Dudgeon, 2016). On Borneo Island there have been only a few studies that have evaluated the biodiversity and community structure of macroinvertebrates in rivers and streams (However see: Dolný *et al.,* 2011; Iwata *et al.,* 2003). This lack of research is combined with high anthropogenic activity including large-scale land use change on the island, which has lead to environmental degradation on a large scale (White and Klum, 2008) . This highlights the immediate need for baseline studies in non-impacted areas.

Rivers provide numerous important ecosystem services, many of which are supported by macroinvertebrates (Wotton and Malmqvist, 2001; Giller and Malmqvist, 1998). These animals are the backbone of riverine ecology and provide the food base for fish and mammals. Macroinvertebrates are important in the cycling of organic matter and controlling periphytic algae and biofilm growth (Wotton and Malmqvist, 2001; Gessner *et al.,* 1999), playing an important role in transferring energy up the trophic levels (Petersen and Cummins, 1974). In terms of riverine management, macroinvertebrates are useful in understanding biodiversity patterns as their distribution often reflects the pattern of food resources and habitats (Ramirez *et al.,* 1998). In addition, their sensitivity to changes in benthic resources and pollution events makes them useful in bio-monitoring giving an insight into any changes in the environment (Wotton and Malmqvist, 2001).

Due to the importance of macroinvertebrates in river ecosystems, it is important to understand natural patterns of these animals within the river network and what factors can control their distribution (Brierley and Fryirs, 2013). It has long been reported that the physical environment can largely determine macroinvertebrate distribution such as changes

in channel width, depth and substrate with distance downstream (Rice *et al.,* 2001). The two main rivers that flow through the Ulu Temburong National Park are Sungai Temburong and its tributary Sungai Belalong (Fig 5.1). These main rivers flow between steep banks, about 35 m in elevation (Orr, 2001), with both Temburong and Belalong composed of a mix of meandering pools and turbulent and fast flowing riffles. Substrate consists of a mix of coarse cobbles and gravel, with many sand/silt bars along the rivers positioned behind debris dams.

Figure 5.1 *Sungai Temburong during low flows*

Daily weather in the Ulu Temburong National Park is very erratic with rainfall driven by convection cells, which produce intense storm events when they rise and condense over the mountains of Ulu Temburong National Park (Cranbrook and Edwards, 1994). The Brunei Hydrometric Unit 483 used to be located near to Kuala Belalong Field Study Centre (KBFSC) has not been in operation for many years after it was scoured out during a flood event. However, recordings taken in the early 1900s during a Royal Geographical Society (RGS) fieldwork survey recorded base flow stage height at approximately 1.2 m and the wet season base flow was ~2m (Cranbrook, 1993). They recorded that a height of 2.54m is approximately a discharge of 36.6 m3 s-1 and a height of 2.3 m is a discharge of 25 m3 s-1. During storm events it is not uncommon for the river to rise 1 m in 30 minutes and during the RGS expedition a 1.2 m rise in 10 min was recorded (Earl of Cranbrook, 1993). Figure 5.2 shows a photo of Sungai Belalong during low flow and the second photo after

approximatly 2 hours of rainfall. Temburong is a larger basin but the hydrological conditions has not been recorded.

Figure 5.2 *Photo of Sungai Belalong outside KBFSC. Photo on the left taken at 17.45 when the rain started and photo on the right taken at 20:00*

This study was conducted around the confluence of Sungai Temburong and Sungai Belalong, creating an opportunity to investigate macroinvertebrate biodiversity and community structure between two tropical rivers. In this study the diversity and distribution of benthic macroinvertebrates are investigated in the pools of Sungai Temburong and its tributary Sungai Belalong. It was not possible to sample the riffles due to the turbulent flows. It was hypothesised that pool diversity would be lower in Temburong compared to Belalong due to its likely higher discharge and potential for increased scouring flood events.

5.2 Methods

5.2.1 Study sites and fieldwork

Fieldwork was conducted on Temburong and Belalong in June 2014 during low water levels making the rivers accessible and safe. Sampling was conducted on 6 pools in Temburong (3 above and 3 below the confluence with Belalong) and on 6 pools in Belalong (all above the confluence; Fig 5.3). To reduce local-scale variability, the study focused on slow flowing biotopes (pools). A standard, D-frame kick net with a 500 micron mesh bag was used to sample the benthic macroinvertebrates. At each site, a 3 minute kick sample of approximately 10m of the river was taken. This ensured all the functional habitats in each pool were included in the sample. Shrimp were not included in this study as they are not effectively sampled with a kick net in these riverine habitats (Jacobsen *et al.,* 2008).

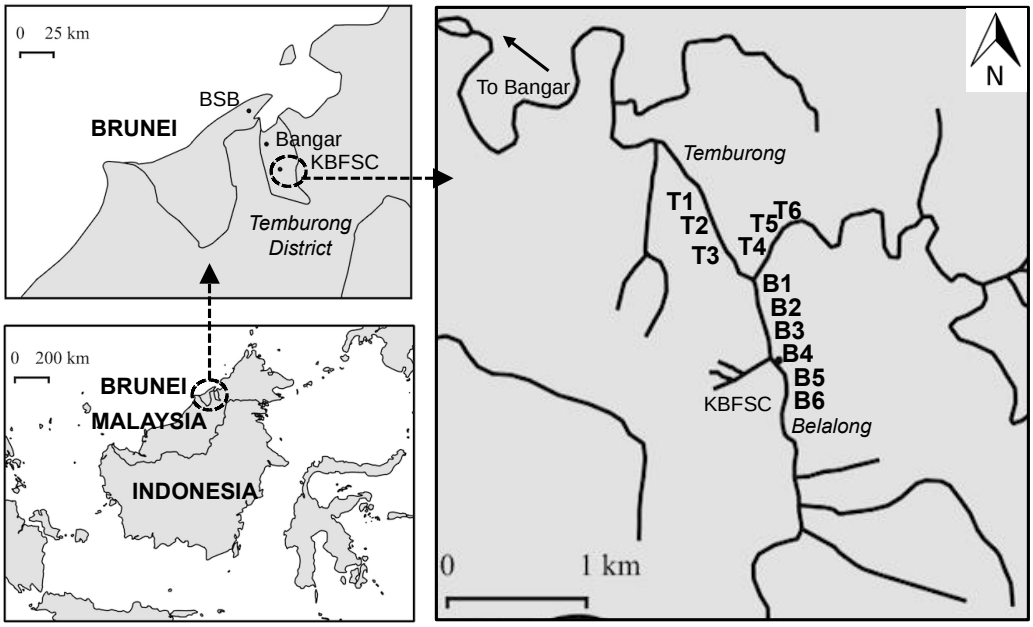


Figure 5.3*. Map of Brunei situated in the north of Borneo Island. The country is split into two parts, with Ulu Temburong National park within the Temburong district. Kuala Belalong Field Study Centre (KBFSC) is situated upstream from the Temburong- Belalong confluence. Kick samples were taken downstream from the Temburong-Belalong confluence (T1-3) and upstream from the confluence on Temburong (T4-6) and Belalong (B1-6).*

5.2.2 Sampling and Identification of Benthic Macroinvertebrates

After collection macroinvertebrate samples were preserved in 70% ethanol and processed under (10x) magnification at the Field Study Centre. Samples were exported to the UK, where they were identified to the lowest practical taxonomic level (genera or morphotyped to a assumed similar level), enumerated and measured to the nearest 0.5 mm. Identifications were conducted using the few keys available including Dudgeon (1999), Yule and Yong (2004), and Sangpradub Boonsoong (2006.) alongside open source identification methods. Specifically, taxa were photographed and uploaded onto the Flickr website (http://flickr.com/photos/tropical-streams/sets/), where interested experts could comment or request to see the actual specimens.

To calculate biomass, taxa were measured to the nearest 0.5 mm, with taxa specific Ash free dry mass (AFDM) being calculated using length-mass regressions (Benke *et al.,* 1999; Sabo *et al.,* 2002; McNeely *et al.,* 2007). When no taxa-specific equations exist, estimates were made using equations from taxa with similar body shape (Ramírez and Pringle, 1998). Where only dry mass (DM) estimates are available, values were converted to AFDM following Waters (1977).

5.2.3 Data Analysis

Macroinvertebrate richness, density and, biomass (AFDM) was quantified at each site. A One Way analysis of variance (ANOVA) was used to test differences in biodiversity, with the data meeting statistical assumptions of normality and homoscedasticity (Thomas *et al.,* 2013). Analysis of similarities (ANOSIM) was used to test if there was a significant difference between taxa in the two rivers, and in sites above and below the confluence on Temburong (Clarke and Warwick, 2001). A statistic called R, which ranges from – 1 to + 1, measures the distinctiveness of the grouping in ANOSIM. Values close to 1 indicate high similarity, 0 indicate that there is little relationship to the groups and -1 indicate samples are outside all of the groups. Assemblage structures were examined using a hierarchical cluster analysis carried out based on the Bray Curtis Coefficient (Thomas *et al.,* 2013). Bray-Curtis similarity matrices calculated the compositional dissimilarity of sites based on the abundance of taxa at each site. Abundance data was used instead of biomass as it had a lower stress number (Stress: 0.15 compared to 0.18 for biomass). Multi-dimensional scaling (MDS) analysis was used to test the robustness of groups defined by the cluster analysis. A sample-size-based rarefaction and extrapolation analysis was carried out to assess the accuracy and completeness of my sampling (Gotelli and Colwell, 2011). Analysis was carried out in the computing environment R (R Core Team 2013).

5.3 Results

5.3.1 Representative sampling

In my study I established a total of 36 taxa in Temburong and 46 taxa for Belalong, both streams had 6 pool replicates (Fig 5.4A). If I doubled my sampling effort to 12 replicates, species diversity at Temburong is expected to increase to approximately 50 taxa and at Belalong to approximately 65 (Fig 5.4A). However, if I eliminate the rare taxa (classed in this study as taxa occurring only once), it shows that I have collected most of the common taxa (Fig 5.4B). These results enabled me to evaluate the community structure of macroinvertebrates in Temburong and Belalong.

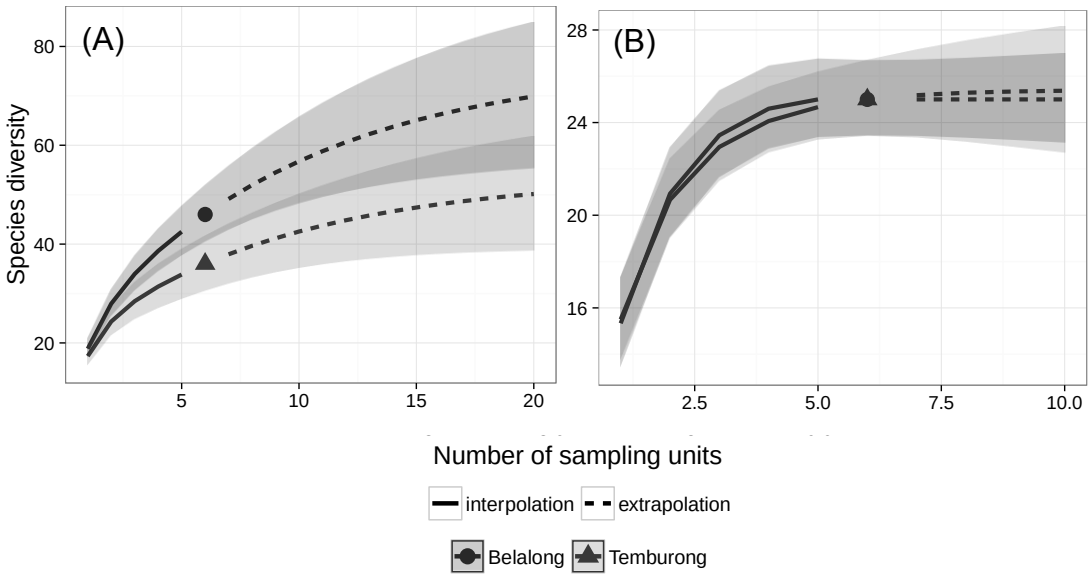


Figure 5.4. *Sample-size-based rarefaction and extrapolation for fast and slow biotopes. Graph (A) includes all sampled taxa and (B) includes the common taxa which is defined as any taxa that is found more than once.*

5.3.2 Temburong and Belalong

A total of 1,184 individuals were collected from the 12 study sites on Temburong and Belalong representing 55 genera from 36 families and 8 orders (Appendix Chapter 5). There was no significant difference in richness found between Temburong (36 taxon) and Belalong (46 taxon; $F_{1,10}$=0.4, p= 0.5257; Fig 5.5). Average abundance was similar between Temburong (93 ± 23 individuals) and Belalong (101 ± 26 individuals; $F_{1,10}$=0.04, p= 0.8; Fig 5.5). Temburong had an average biomass of 25.11 ± 5 mg and Belalong had 24 ± 7 mg, with no significant difference between the two rivers ($F_{1,10}$=0.008, p=0.9; Fig 5.5).

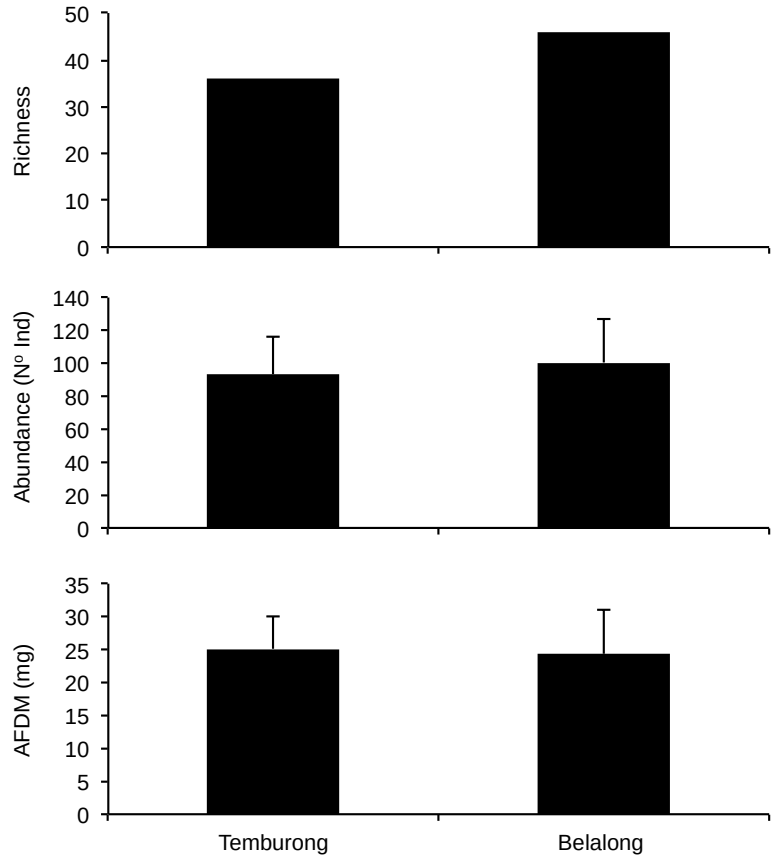


Figure 5.5*. Total richness, density, and biomass (ash-free dry mass; AFDM) of macroinvertebrates for Temburong and Belalong. Error bars represent standard deviations (Temburong, n = 6; Belalong, n = 6).*

The hierarchical cluster analysis showed no clear difference in community structure between the rivers (Fig 5.6). While the NMDS ordination (stress: 0.15) of benthic macroinvertbrate abundance showed a slight divide in community structure between Temburong and Belalong (Fig 5.6). ANOSIM results have an R value of 0.35 and a significance level of p=0.06, suggesting there is little difference in the macroinvertebrate communities between the rivers. The results do show that the hierarchical cluster analysis has a 0.6 dissimilarity between pools above and below the confluence on Temburong (Fig 5.6). An ANOSIM analysis also shows a difference in macroinvertebrate communities in the Temburong pools above and below the confluence with Belalong (R value: 0.59 and significance level of 0.01). However, there was no difference in richness ($F_{1,4}$=2.88, p=0.16) or biomass($F_{1,4}$=2.11, p= 0.22), and a difference in abundance ($F_{1,4}$= 8.12, p= 0.05). Pools downstream of the confluence had an average relative abundance of 51 individuals whereas average relative abundance in pools above the confluence was 136 individuals.

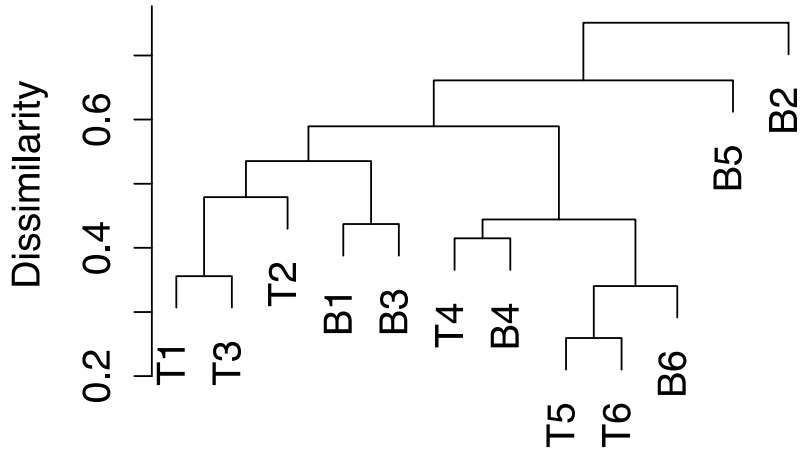


Figure 5.6. *Dendrogram of benthic aquatic insect abundance at each of the sites. T=Temburong and B=Belalong. Numbers indicate replicate pools.*

Ephemeroptera was the most dominant order at both Temburong (66% of total abundance) and Belalong (58% of total abundance; Appendix Chapter 5). Euthraulus (Family: Leptophlebiidae) was the most abundant taxa in the study with a total relative abundance of 338 individuals, of which 99 individuals were found at Temburong site 4, the first site upstream from the confluence with Belalong (Fig 5.7). In contrast there were zero Euthraulus present at Belalong site 2. Other taxa from the order Ephemeroptera were abundant. Including Potamanthus (Family: Potamanthidae) with 124 individuals sampled in the study (85 of which where found at Belalong site 5) and the flattened Mayfly Compsoneuria (Family: Heptageniidae) had 107 individuals. At the other end of the scale there were some taxa with very low relative abundances, for example 14 macroinvertebrates only occurred once in the survey and 35 taxa had a total abundances of less than 5 (Appendix Chapter 5).

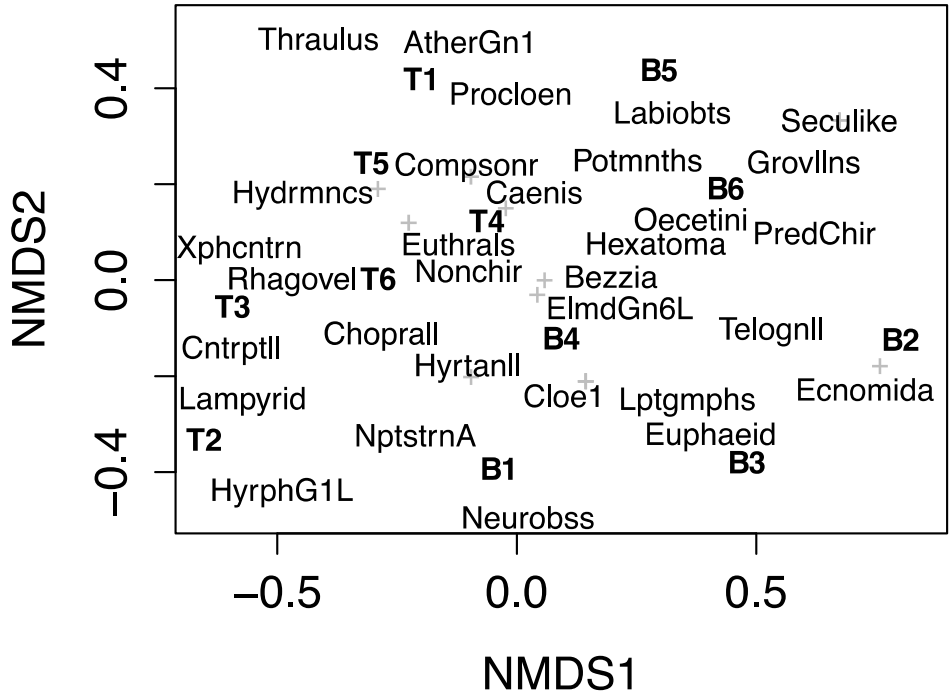


Figure 5.7. *NMDS of macroinvertebrate community patterns and sampling sites. The light grey crosses represent taxon that are less than the mean, only the priority taxon (greater than the mean) are displayed. List of taxa abbreviations are in appendix Chapter 5. The dotted line provides a subjective division between the Temburong and Belalong pools. (Stress: 0.15).*

5.4 Discussion

In any environment it is difficult to collect representative samples of nature (Hsieh *et al.,* in press; Chao *et al.,* 2014). However, this is accentuated in the tropics where there is high taxa diversity but with many rare taxa (Gotelli and Colwell, 2011). In my study I found many taxa only occurred once, with other taxa occurring at very low abundances (Appendix Chapter 5). The slowly rising taxa accumulation curve in Fig 5.4A is not uncommon for data from the tropics with biodiversity surveys frequently missing taxa due to the high number of rare taxon (Gotelli and Colwell, 2011). Bishop (1973) suggests that macroinvertebrate population densities are low in tropical streams as a result of the constant, but unstable microhabitats caused by frequent flood events, scouring out functional habitats. However, despite the likely high number of rare taxa present in my study sites, the rarefraction curve does show that I have sampled most of the common taxa (Fig 5.4B). It is important to note that these common taxon that will be important in understanding biodiversity patterns in tropical streams.

This study recorded only 36 families with a total of 55 genera (36 at Temburong and 46 at Belalong). Similar studies in the tropics have reported higher levels of taxon richness,

for example in the Liwagu river catchment (Sabah, northern Borneo) 61 families were recorded (Mazlan and Kamarudin, 2015), and Bishop (1973) found 204 morphospecies during his longitudinal survey of Sungai Gombak (Peninsular of Malaysia). However, Bishop's (1973) study involved sampling the whole river therefore it would be expected to have higher taxon richness. The relatively low level of richness in my study could be a result of a focus on just one biotope, the pools (Mac Nally *et al.,* 2011). Interestingly, macroinvertebrate diversity in Temburong and Belalong were low compared to pool taxon richness in their tributaries (chapter 7), with 37 taxa were reported at Sungai Lower Apan (tributary of Temburong with 6 pools sampled), 50 taxon at Sungai Esu (tributary of Belalong with 5 pools sampled) and 61 taxon at Sungai Threelan (tributary of Temburong with 6 pools sampled).

The low levels of richness in Temburong and Belalong could be explained by the frequent and high intensity precipitation events that cause water levels to rise in a short period of time. The deep 'V' shaped valleys prevent the river from expanding laterally causing the discharge to increase further. Although it was not possible to monitor river discharge, there is descriptive evidence of the river's power during flood events. For example during flood events the river is capable of carrying large tree trunks, which then accumulate and create wood debris dams after peak flow (Cranbrook and Edwards, 1994). These debris dams can be on an impressive scale, for example in between two fieldwork trips from 2013 to 2014, a debris dam tens of meters in size was washed downstream during a large storm event (Fig 3.7). In comparison the tributaries of Temburong and Belalong, including Lower Apan, Esu and Threelan, had less visible movement of large boulder and debris dams in between fieldwork trips (2012-14; personal observations). These smaller tributaries have smaller catchments and therefore less volumes of water entering during storm events, which appear to reflect in higher levels of macroinvertebrate biodiversity (Chapter 7).

In both Temburong and Belalong, Ephemeroptera comprised more than >50% of the taxa. Comparisons to other studies in the region found a more diverse range of orders. For example a similar study in Tambunan (Sabah, Malaysia) found the dominant taxa was 20% Trichoptera, 18% Coleoptera followed by 16% for Ephemeroptera (Mazlan and Kamarudin, 2015). Aquatic insects in the streams of Mae Klong watershed (Thailand) also found a more diverse mix of macroinvertebrates with Trichoptera dominating (~35%) followed by Ephemeroptera (~20%) and Odonata (~10%) (Maneechan and Prommi, 2015). The similarity of macroinvertebrate communities between the study rivers may be a result of

similar scouring flows, which play an important role in structuring aquatic communities (Bishop, 1973; Peckarsky *et al.,* 1990, Yang and Dudgeon, 2010). Sheldon (2011) suggests the streams and rivers of Ulu Temburong were physically controlled by the frequent flood events, with biotic interactions between grazing fish being minimal. The most abundant taxa in this study have evolved to withstand fast flows including Euthraulus that take shelter in bottom sediments or tailing roots, Potamanthus are adapted to burrowing, and Compsoneuria have depressed bodies (Ghee, 2004).

The similar macroinvertebrate communities in Temburong and Belaong may also be a result of hydraulic damming and 'back up' of Belalong, this occurs when there is a flood event on Temburong and the river starts flowing up Belalong. This would carry macroinvertebrates from Temburong in its drift. This type of migration has been recorded in the River Rhine, with Macroinvertebrate dispersal occurring upstream during a flood (Beckmann *et al.,* 2005). Dispersal can also occur over short distances during base flow (Elliott, 2003). Interestingly, results show there is a higher similarity in macroinvertebrate communities between Temburong and Belalong, than compared to Temburong pools situated above and below its confluence with Belalong. Differences in biodiversity have been found to change after a confluence, with macroinvertebrate abundance and diversity often increasing downstream (Wallace *et al.,* 1991; Rice *et al.,* 2001; Knispel and Castella, 2003; Benda *et al.,* 2004). However, my results have found a decrease in macroinvertebrate abundance below the confluence (51 individuals below confluence compared to 136 individuals in pools above the confluence). The physical effect of the tributary on the main river, such as scour and change in benthic substrate, occurs immediately downstream of the confluence (Best, 1988; Wallis *et al.,* 2008). This may be particularly high in the Temburong catchment due to the large volumes of water that meet at the confluence, which causes high levels of scouring, washing out functional habitats and physically removal invertebrates. Conversely, the hydraulic damming that occurs on Belalong could reduce the river velocity and consequence of extreme scouring.

5.5 Conclusion

Results from this study show there is no difference in biodiversity or community structure between the pools in Temburong and its tributary Belalong. However, there were differences in macroinvertebrate abundance and community structure between pools upstream and downstream of the confluence on Temburong. On a local scale, the collection

of baseline data in the Temburong catchment is important to monitor and manage the river effectively, both within and outside the national park where anthropogenic factors including agriculture, and urbanisation can have negative impacts on the river's biodiversity. By having a broad understanding of the river's benthic community, it may be used to help mitigate any unnecessary impacts on the river's biodiversity. This dataset is the beginning for creating benchmarks for understanding tropical rivers in Asia and sets the scene for the subsequent chapters in this thesis, which focus on the tributaries of these rivers.

Chapter 6: The Effect of River-Tributary Interactions on Benthic Habitat & Macroinvertebrate Biodiversity

6.1 Introduction

Confluences are frequently described as ecotones, a concept originally coined by plant ecologists Livingston (1903) and Clements (1905), as a boundary area among communities where there is an exchange between two biotopes or habitats (Amoros *et al.*, 1996; Hubbel and Ryan, 2016). For river networks, these ecotones can have high levels of habitat heterogeneity due to the increased variation in physical conditions (Todorova and Topalova, 2014). Further, given the abrupt change in the river's continuum over short distances (i.e., metre rather than kilometres) a wide range of environmental conditions within these boundary areas can promote increased levels of biodiversity relative to the intersecting systems (Amoros *et al.*, 1996). Despite the general understanding of these dynamics (Rice *et al.,* 2008a), the ecology of river confluences has been overlooked with any changes in the river continuum being classed as interruptions rather than important parts of the stream network (Vannote *et al.,* 1980; Bruns *et al.*, 1984; Minshall *et al.,* 1985).

Many physical parameters of the rivers adjust abruptly at confluences and as a result changes in channel widths and depths, substrate type and size, and temperature are frequently observed (Benda *et al.*, 2004; Rice *et al.*, 2008b; Mac Nally *et al.*, 2011). Increases in hydraulic power where two channels converge, especially during spates, often cause scouring which erode channels, exporting larger particle sizes and can result in increased channel widths and depths. However, at these locations in river networks there also tends to be a decrease in river bed slopes leading to pool formations with reduced river flows and particle deposition. The larger substrate sizes are deposited first as the river's energy reduces, with the finer sediment being carried further. Thus, the buildup of substrate at confluences can lead to the development of depositional bars and fans (Bull 1977; Fig. 6.1). These areas of deposited substrate can be permanent or transient, depending on the rate at which the sediment is transported down from tributaries and then scoured by main river discharges. Benda *et al* (2004) argues the degree of confluence habitat heterogeneity is dependent on the disturbance regime of the catchment including rainfall frequency, and geomorphic processes, such as landslides.

Figure 6.1. *Substrate accumulating confluence zone 8 (photo on the left) and organic matter at confluence zone 7 (photo on the right)*

The physical changes that occur at river-tributary confluences can in turn affect the quantities and distribution of organic matter and periphyton. Organic matter which is deposited in tributary channels can be transported downstream and accumulate along with deposited substrate at the confluence (Cranbrook and Edwards, 1994; Wallis *et al.*, 2008; Fig. 6.1). In locations where larger wood and boulders are transported, major changes in geomorphology can promote the formation of debris dams and associated pools, which further promote the accumulation of organic material (Montgomery, 1999; Benda *et al.*, 2004). For periphyton, light limitations in tributaries can occur due to dense canopy cover and/or shading from steep banks. These conditions frequently depress primary production and suppress algal standing stocks (Hill *et al.,* 1995*;* Young and Huryn, 1999; Kiffney *et al.*, 2006). However, breaks in the canopy which can also occur at the confluence increase light levels and facilitate primary production and periphyton growth (Power and Dietrich 2002; Kiffney *et al.*, 2006).

The unique hydrology, geomorphology and organic matter dynamics of river-tributary confluences play a major role in structuring aquatic communities at both the local and landscape scale (Osborne and Wiley, 1992; Benda *et al.*, 2004; Rice *et al.*, 2008a). Defined as "biodiversity hotspots" due to the higher diversity of macroinvertebrates (Knispel and Castella, 2003; Rice *et al.*, 2001), fish (Fernandes *et al.*, 2004; Kiffney *et al.*, 2006), and even larger aquatic animals such as river dolphins (McGuire and Winemiller, 1998). The importance of confluences have even been extended to terrestrial fauna, with bird abundance and diversity found to peak near to confluences due to the extra food resources (Rice *et al.*, 2008a). During flood events confluences and tributaries can act as areas of refuge from

scouring flows (Wallis *et al.*, 2008), while during low flows confluences can be vital habitat being deeper compared to the rest of the river network (Wallis *et al.*, 2008). Understanding the distribution of macroinvertebrates in the confluence zones may be useful in appreciating the utility of confluences, as their distribution often reflects the pattern of food resources and habitats (Ramirez *et al.,* 1998).

Ulu Temburong National Park in Brunei Darussalam, where this study was conducted, experiences very localized and intense rainfall events, with no month described as "dry" (Dykes, 1997). For example, during the same day 63.5 mm of rain fall was recorded at Kuala Belalong Field Study Centre (KBFSC) but less than 10km away, only 19 mm was recorded (Cranbrook and Edwards, 1994). Water levels are known to rise in a short space of time and the localization of the storms can set up extreme differences between the main river flooding and the base flows in the downstream tributaries (Fig. 6.2). During low flows there is little interaction between the main river and tributaries. Whereas after a storm event the main river expands in size and with increasing discharge a process called 'hydraulic damming' can occur, preventing tributaries from flowing into the main river. During large storm events the main river even flows up the tributaries (Baker, visual observations), only stopping when reaching a waterfall or steep cascade (Fig. 6.2). This process is accentuated due to the steep banks that occur on Sungai Temburong and Belalong (Orr, 2001), which limits any flood plain development.

To date most confluence studies have focused on the effects of tributaries on changing macroinvertebrate communities in the main river (Bruns *et al.*, 1984; Stevens *et al.,* 1997; McKie, 2004; Rosales *et al.*, 2007). By contrast, there has been few studies that have focused on how the main river affects macroinvertebrate communities on its tributaries. Beckmann *et al* (2005) study on the River Rhine and its tributaries found macroinvertebrates and benthic substrate at the tributary river margin sites were affected by higher levels of velocity, increased sedimentation and more variable environmental conditions compared to biotopes upstream in the tributary. Wilson and McTammany (2014) also found that the river affected macroinvertebrate communities on its tributaries, which was suggested to be due to direct dispersal and indirect habitat alteration. In the tropics, hydraulic damming has been reported on redundant channels connected to floodplains, for example on the Orinoco River in Venezuela a backwater effect of more than 50m has been observed during flood events (Herrera and Rondon, 1985). However, there have been no recorded studies conducted on tributaries that experience hydraulic damming on a frequent basis. Ulu Temburong National

Park is an excellent study site to conduct this study due to the rivers having steep banks with no floodplain and high rainfall events, which cause the main river to expand at confluence zones and flow upstream the tributaries.

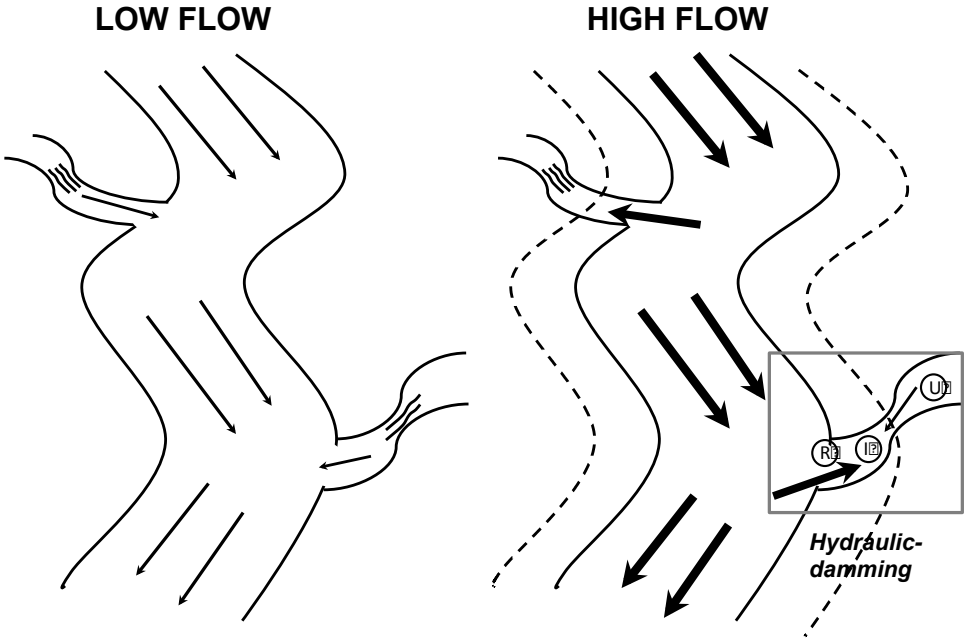


Figure 6.2. *Schematic to show how a changes from low (left schematic) to high flows (right schematic) causing hydraulic damming and back-up. Low flow channel-width is in dark black with high flow channel-width in dashed black. Arrows represent the water flow direction and thickness of the arrow represents level of discharge. Water can rise and fall within hours. During low flows, water flows out of the tributaries into in the main rivers and during high flows the main river can block the tributary flow or cause hydraulic damming. Sampling sites are highlighted as the river margin (R), river-stream interface (I) and upstream pool (U) above a high gradient riffle or waterfall where the main river only interacts during a significant storm event. The box in grey highlights the confluence zone.*

The goal of this study was to investigate the influence of the main rivers in Ulu Temburong National Park (Sungai Temburong and Sungai Belalong) on their tributaries. Specifically, the work focused on the confluence zone including sites at the river margin (R), the river-stream interface (I) and the upstream pool (U) which is above a high gradient riffle or waterfall where the main river only interacts during a significant storm event (Fig. 6.2; grey box). At each of the 11 confluence zones, I assessed substrate, organic matter (leaf litter and wood debris), and standing stocks and growth rates of periphyton. In addition, the diversity and community structure of macroinvertebrates were evaluated. It was hypothesised that the highest levels of organic material and periphyton would be at the river-stream interface, which will consequently increase diversity of macroinvertebrates.

6.2 Methods

6.2.1 Study Site

Ulu Temburong National Park in the Temburong district of Brunei Darussalam is covered in primary rainforest with an undisturbed river catchment, a landscape that is becoming increasingly rare on Borneo Island (Sheldon, 2011; Bryan *et al.*, 2013). The national park has a sharp topography, the Temburong-Belalong confluence is 30 m a.s.l. but rises to 913 m a.s.l. (9 km away) at Bukit Belalong and to 1,850 m a.s.l. (35 km away) at Bukit Pagon (Dykes, 1994). Brunei Darussalam has a tropical climate and although it is influenced by the annual climate pattern of the South East Asian monsoon, the daily weather in the national park is normally very erratic (Dykes, 1996). Rain usually originates as convection cells, as these cells rise over the mountains of Ulu Temburong, they condense and produce rain (Cranbrook and Edwards, 1994).

6.2.2 Study design

At each confluence zone, substrate, organic matter, and macroinvertebrates were sampled at the main river margin (M), river-stream interface (first pool upstream from the river margin; I) and an upstream pool (above a high gradient riffle or waterfall where there is little interaction with the main river; U; Fig. 6.2). To reduce local-scale variability only slow flowing biotopes (pools) were sampled. Pools are found in every tributary and are uniformly similar in terms of substrate, velocities and depths compared to fast flowing biotopes (riffles, cascades and waterfalls). Eleven confluence zones were selected for this study, all situated on tributaries within a 3 km radius (Fig 6.3). Four were tributaries of Sungai Belalong (1, 2, 3 and 11), three tributaries of Sungai Temburong upstream of the Sungai Temburong-Belalong confluence (7, 8 and 9) and four were tributaries of Sungai Temburong downstream of the confluence (4, 5, 6 and 10; Fig 6.3). All of the confluence zones had either a high gradient riffle or waterfall upstream which enabled me to sample a pool that was not influenced directly by hydraulic damming caused by the main river. Three of the tributaries (6, 10 and 11) had waterfalls directly upstream of the river margin, therefore only the river margin and upstream pool was sampled.

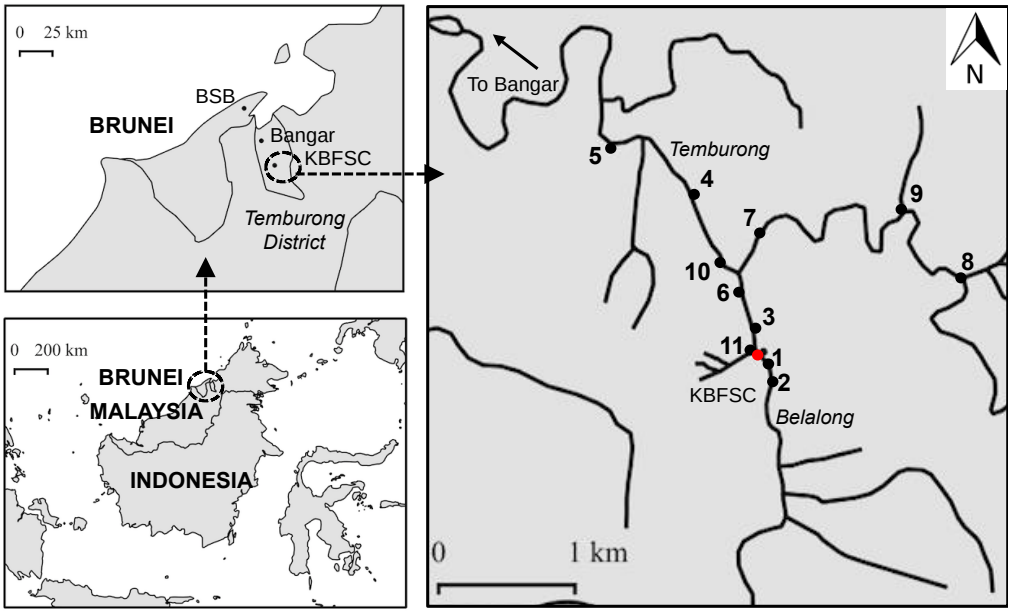


Figure 6.3. *Brunei is situated in the north of Borneo Island. The country is split into two parts, with Ulu Temburong National park within the Temburong district. Kuala Belalong Field Study Centre (KBFSC) is highlighted by the red dot and is situated on the banks of Sungai Belalong. The confluence zones included in this study are numbered 1-11 and identified by black dots.*

6.2.3 Field Methods

Physical habitat measurements: To establish variation among the confluence zones and to categorise tributary size, velocity and depth was taken at each of the tributary confluences every 0.5 m following common methods described by Gorden *et al.,* (2004). Wetted widths (defined by the area of stream channel filled with water) was also recorded. Channel dimensions were measured with surveying tapes and meter sticks. Stream velocity was measured at 60% depth (Gordon *et al*., 2004), using an electromagnetic flowmeter (Valeport® model 801; Valeport Ltd., Totnes, UK). Substrate type at each location was assessed visually and categorised as percent silt and sand, gravel, cobble, boulder and bedrock at the river edge, confluence and tributary.

Organic matter: Organic matter was collected at the river margin (R), river-stream interface (I) and upstream pool (U). A quadrat was placed on the riverbed and all of the organic material inside was sampled. The organic material was brought back to the KBFSC and dried at 70ºC for 24 hours. The material was divided by eye into three categories; leaf litter, wood and 'other' material composed of amorphous material. Ash Free Dry Mass (AFDM) was calculated by taking the difference between the dry weight and ashed weight (500ºC for 2 hr). AFDM summed across all categories was then used as an estimate of total organic matter. A one way Analysis of variance (ANOVA) was used to test for statistical

differences in organic matter among the 11 confluence zones. Further, differences among the 3 sampling locations (R, I and U) pooled across confluence zone were evaluated. All data was transformed (SQRT+1) to minimise deviations from normality and homoscedasticity.

Periphyton: Due to time constraints associated with the remote location of this research, periphyton levels were estimated from a random sub-sample of confluence zones (1, 3, 8 and 10). Random rocks were selected for the experiment from the streams in Ulu Temburong National Park, placed in boiling water and scrubbed to remove all periphyton. Rocks were then placed and secured at the river margin and in the upstream pool with plastic wire. During the experiment, there were some periods when the water levels dropped causing a few of the experimental rocks to be exposed out of the water. As this was for short periods of time, they were still included in this study. This occurred at tributary 1 (confluence and upstream pools), tributary 3 (confluence pool) and tributary 10 (confluence). After exposure for 47 days, a fixed area of (0.002m^2) of each rock was sampled with the periphyton collected on GFF filters. The filters were dried at the KBFSC and transported back to the UK for Ash Free Dry Mass (AFDM) analysis to be conducted. AFDM was calculated by taking the difference between the dry biofilm weight and ashed weight (500ºC for 1hr). AFDM was then used as an estimate of periphyton standing stock levels. Growth rates were then estimated as AFDM divided by exposure time. This method of periphyton estimates cannot distinguish between living and dead algal cells (Wellnitz and Poff, 2006), but was selected due to remote locations and limited facilities at the KBFSC. I assumed that the accumulation of periphyton would likely approximate linear after the initial exponential colonization stage. Standing stocks of periphyton in each of the study confluence zones were quantified from four random rocks at the river margin and the upstream pool to ensure periphyton growth levels from the experiment were comparable with background standing stock. One way Analysis of variance (ANOVA) was used to test for statistical differences in ambient periphyton and periphyton growth rates among the 11 confluence zones. Differences in periphyton were also assessed among the 3 sampling locations (R, I and U) pooled across the confluence zones. The ambient periphyton was transformed (SQRT+1) to minimise deviations from normality and homoscedasticity.

Macroinvertebrate: A three-minute kick sample (standard D-frame with 500µm mesh bag) was used to collect benthic macroinvertebrates in the study pools at the river margin, river-stream interface and upstream pool. Shrimp are not effectively sampled by kicks in these habitats (Jacobsen *et al.,* 2008), therefore the results for these taxa are at best an

estimate. Due to the large size of shrimp compared to other macroinvertebrates they were treated separately for biodiversity calculations (richness, abundance, and biomass). In the field, samples were preserved in 70% ethanol. In the laboratory macroinvertebrates were processed under (10x) magnification and identified to the lowest practical taxonomic level (genera or morphotyped to a similar level) and measured to the nearest 0.5 mm. Due to the lack of macroinvertebrate taxonomic knowledge in the tropics, open source identification methods with tropical specialist taxonomists was conducted alongside using the few keys available (Dudgeon 1999; Yule and Yong, 2004; Sangpradub *et al.,* 2006). At 4 sampling sites the identification of shrimp down to genera or even family level was not possible due to the small sizes of collected specimens therefore in the results shrimp were combined together as one taxa. AFDM of macroinvertebrates was estimated using length-mass regressions (Benke *et al.,* 1999*;* Sabo *et al.,* 2002*;* McNeely *et al.,* 2007). In cases were no associated equations were available, estimates were made using taxa with similar body shape (Ramírez and Pringle, 1998).

To assess macroinvertebrate biodiversity, total richness, density, and biomass (AFDM) were quantified. ANOVA was used to test for any statistical differences in biodiversity between the tributaries, and kick samples. To ensure that data was normally distributed macroinvertebrate biomass and abundance data for the kick samples was transformed (SQRT+1). Similarity percentage analysis (SIMPER) was used to identify the taxa that contributed most to the average dissimilarity among the pools (R, I and U) and between the confluence zones. Analysis of Similarities (ANOSIM; Clarke, 1993) was used to test for differences in composition of macroinvertebrates communities among the pools (R, I and U). The global R statistic, which ranges from -1 to +1, measures the distinctiveness of the grouping according to ANOSIM. Values close to 1 indicate high similarity among groups, 0 indicates that there is no relationship in composition among the groups, and -1 indicates samples are distinct to each group. Abundance data were used for both SIMPER and ANOSIM, and both of these tests use the Bray–Curtis index, a popular dissimilarity index for ecological data (Borcard *et al.,* 2012). Although rare taxa can be important in community structure, in the SIMPER analysis I only included percentage contribution of more than 4 %.

6.3 Results

6.3.1 Physical measurements of confluence zones

Average tributary channel depths varied from 0.03m (confluence zone 10) to 0.65m (confluence zone 5; Table 6.1). Velocities varied from -0.14 ms^{-1} (i.e., river flows going into the tributary from the main river; confluence zone 3) to 0.68 ms^{-1} (i.e., tributaries flowing into the main river; confluence zone 11; Table 6.1). Average velocities tended to be higher at confluence zones 6, 10 and 11 as these had waterfalls at the stream-river interface, whereas the other confluence zones had pools. During fieldwork, average discharges were near to zero, but in any given site there was measurable water moving downstream. Although the averages for some locations were negative, there was observable flow from the tributaries to the main river at all sites.

Table 6.1. *Channel characteristics and physical variables of the eleven confluence zones, one measurement was taken at the confluence site.*

Confluence zone	Channel Width (m)	Average depth (m)	Average velocity (m s^{-1})
1	2.2	0.04	0.30
2	2.6	0.08	0.12
3	3.8	0.26	-0.18
4	2.3	0.40	-0.08
5	4.0	0.65	-0.11
6	2.6	0.07	0.48
7	2.1	0.22	-0.07
8	10.6	0.23	0.09
9	6.2	0.45	-0.14
10	0.3	0.03	0.54
11	0.3	0.14	0.68

Confluence zones 5 and 6 had the lowest levels of silt and sand (< 10%) and in contrast confluence zone 4 was comprised of 80% silt and sand. The percentage of gravel was more consistent among the confluence zones, while average percentage of cobbles was lowest at confluence zones 11 and 4 (less than 5%) and highest at confluence zone 6 (70%). Bedrock was only present at confluence zone 10. Average substrate at the river margin was composed of sand (~45%) and cobbles (~40%). At river-stream interface it was predominantly sand (~67%) and at the upstream pool the substrate was composed of cobbles (~40%) and gravel (~40%- Table 6.2).

Table 6.2. *Physical measurements at the confluence zone. Including distance from the river margin to the river-stream interface and upstream pools, and the percentage substrate of bedrock (BR), cobbles (C), gravel (G) and silt & sand (S&S). There were no boulders in the confluence zones and there were no river-stream interface sites at confluence zones 6, 10 and 11 due to waterfalls.*

Tributary number	Confluence zone	Distance from river (m)	BR	C	G	S&S
1	River margin		-	-	-	100
	River-stream interface	1.6	-	-	10	90
	Upstream pool	12.3	-	100	-	-
2	River margin		-	40	-	60
	River-stream interface	7.2	-	20	20	60
	Upstream pool	16.5	-	90	10	-
3	River margin		-	90	10	-
	River-stream interface	7.8	-	-	10	90
	Upstream pool	12.9	-	20	20	60
4	River margin		-	10	-	90
	River-stream interface	4.8	-	-	10	90
	Upstream pool	6.9	-	-	40	60
5	River margin		-	10	70	20
	River-stream interface	3.6	-	50	40	10
	Upstream pool	13.9	-	60	40	-
6	River margin		-	90	10	-
	Upstream pool	11.1	-	50	50	-
7	River margin		-	10	10	80
	River-stream interface	8.5	-	10	10	80
	Upstream pool	9.2	-	20	80	-
8	River margin		-	80	10	10
	River-stream interface	40.0	-	-	10	90
	Upstream pool	78.2	-	40	40	20
9	River margin		-	100	-	-
	River-stream interface	30.0	-	-	10	90
	Upstream pool	25.0	-	20	70	10
10	River margin		-	-	-	100
	Upstream pool	4.5	50	-	20	30
11	River margin		-	10	70	20
	Upstream pool	8.9	-	20	60	20

6.3.2 Organic matter at the confluence zones

There was a large variation, but no statistical difference in the quantity of organic matter between the confluence zones ($F_{2, 27}$=0.7, p=0.7; Fig. 6.4). The average levels of organic matter varied from 77 ± 53 g AFDM m^2 (confluence zone 5) to a maximum average of 1195 ± 1089 g AFDM m^2 (confluence zone 8). Among the tributaries the quantity of leaves ($F_{2, 27}$=1.2, p=0.3), wood debris ($F_{2, 27}$=0.4, p=0.9) and other material ($F_{2, 27}$=0.8, p=0.6) were not statistically different (Fig. 6.4). Further, there was no difference in total organic matter between the river margin, river-stream interface and upstream pool ($F_{2, 27}$=1.2, p=0.3). Among the three sampling sites the average levels of organic matter varying from 682 ± 392

g AFDM m^2 (river-stream interface) to 169 ± 43 g AFDM m^2 (upstream pool). The quantity of leaves ($F_{2, 27}$=1.13, p=0.3), wood debris ($F_{2, 27}$=1.9, p=0.2) and other material ($F_{2, 27}$= 1.4, p=0.3) were also similar. At the river margin at both confluence zones 7 and 11 there were no leaves. While at the river margin at confluence zones 7, 8, 10 and 11 there was no wood debris.

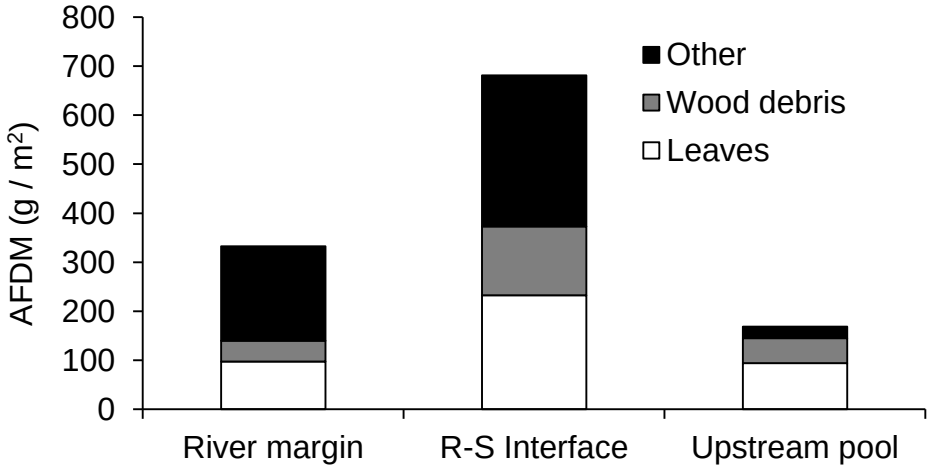


Figure 6.4. *Average AFDM of organic content separated into leaves, sticks/twigs and other/amorphous detritus at the confluence zone (river margin, river-stream interface and upstream pool). No statistical difference were found among these sampling locations. See text for standard deviation (river margin n= 11, confluence n = 8, tributary n = 11).*

6.3.3 Periphyton at the confluence zones

Among the confluence zones ambient periphyton biomass varied from a maximum average of 15.8 ± 7.8 g AFDM m^{-2} (confluence zone 1) to 4.3 ± 0.2/0.7 g AFDM m^{-2} (confluence zone 3 and 10). There was a significant difference in periphyton biomass between the confluence zones ($F1_{3, 19}$=11.5, p=<0.01), explained mainly by the high levels of periphyton at the river margin at confluence zone 1 (Table 6.3). Among the confluence zones average periphyton growth rates ranged from 0.10 ± 0.003/0.01 g AFDM m^{-2} d^{-1} (confluence zone 1 and 10) to a maximum average of 0.24 ± 0.1 g AFDM m^{-2} d^{-1} (confluence zone 3). There was no significant difference in periphyton growth levels between the confluence zones ($F_{3, 17}$=2.3, p=0.1).

Average ambient periphyton biomass was 5.2 g AFDM m^{-2} in the upstream pool and 9.1 g AFDM m^{-2} at the river margin. There was no significant difference in ambient periphyton biomass between the river margins and the upstream pool ($F_{1, 21}$=1.4, p=0.2) and average growth rates were similar between the river margin (0.1 g AFDM m^{-2} d^{-1}; $F_{1, 19}$=0.23, p=0.6) and the upstream pool (0.2 g AFDM m^{-2} d^{-1}).

Table 6.3. *Periphyton growth rate (per day) and ambient periphyton at confluence zone's 1, 3, 8 and 10.*

Confluence zone	Site	Growth rate (g AFDM m^{-2} d^{-1})	Ambient Periphyton AFDM (g m^{-2})
1	Upstream pool	0.10	8.00
	River margin	0.10	23.60
3	Upstream pool	0.35	4.05
	River margin	0.08	4.51
8	Upstream pool	0.08	3.93
	River margin	0.21	4.84
10	Upstream pool	0.10	4.97
	River margin	0.11	3.62

6.3.4 Biodiversity among the confluence zones

In total 123 macroinvertebrate taxa (Appendices Chapter 6a,b&c) were collected in this study, with the highest level of richness at confluence zone 8 (57 taxa) and the lowest at confluence zone 6 (30 taxa). Relative abundance (i.e., individuals per kick sample) varied from an average of 103 ± 47 individuals at the confluence zone 11 to a maximum average of 458 ± 220 individuals at confluence zone 7. The lowest level of biomass was 18.74 ± 11 mg at confluence zone 10 and highest was 100.51 ± 15 mg at confluence zone 9. There was no difference in macroinvertebrate richness ($F_{10, 19}$= 1.12, p= 0.4), abundance ($F_{10, 19}$=2.2, p= 0.1) or biomass ($F_{10, 19}$= 1.8, p=0.12) between the confluence zones. Two genera of shrimp were present in this study including *Macrobrachium* and *Caridina,* however due to some of the small shrimp sizes it was not possible to identify all them to genus level therefore shrimp were analysed together. Confluence zone 6 had the highest relative abundance (30 ± 29 individuals) and biomass (9064.5 ± 9063 mg) of shrimp, the high standard deviations is due to only 1 shrimp being present at the river margin and 58 shrimp present in the upstream pool. There were no shrimp at any of the sites at confluence zone 2. There was no significant difference in relative shrimp abundance ($F_{10, 19}$=1.59, p=0.2) or biomass ($F_{10, 19}$=1.24, p=0.3) between the confluence zones.

6.3.4 Biodiversity at river margin, river-stream interface and upstream pool

Total richness was highest at the upstream pool (96 taxa) followed by the river-stream interface (90 taxa) and the river margin (73 taxa). Average relative abundance was highest at the river-stream interface (278 ± 26 individuals), followed by the river margin (124 ± 21 individuals) and the upstream pool (205 ± 15; Fig. 6.5). Biomass (per kick sample) was highest at the river-stream interface (61.26 ± 6 mg), followed by the upstream pool (42.25 ±

3 mg) and the river margin (32.65 ± 9 mg). There was no difference in macroinvertebrate richness ($F_{2, 27}$=2.2, p=0.13), abundance ($F_{2, 27}$=2.3, p=0.12) or biomass ($F_{2, 27}$=2.3, p=0.12) between the sites, however ANOVA coefficients suggest there are differences in macroinvertebrate abundance (p=0.05) and biomass (p=0.04) at the river-stream interface (Fig 6.5).

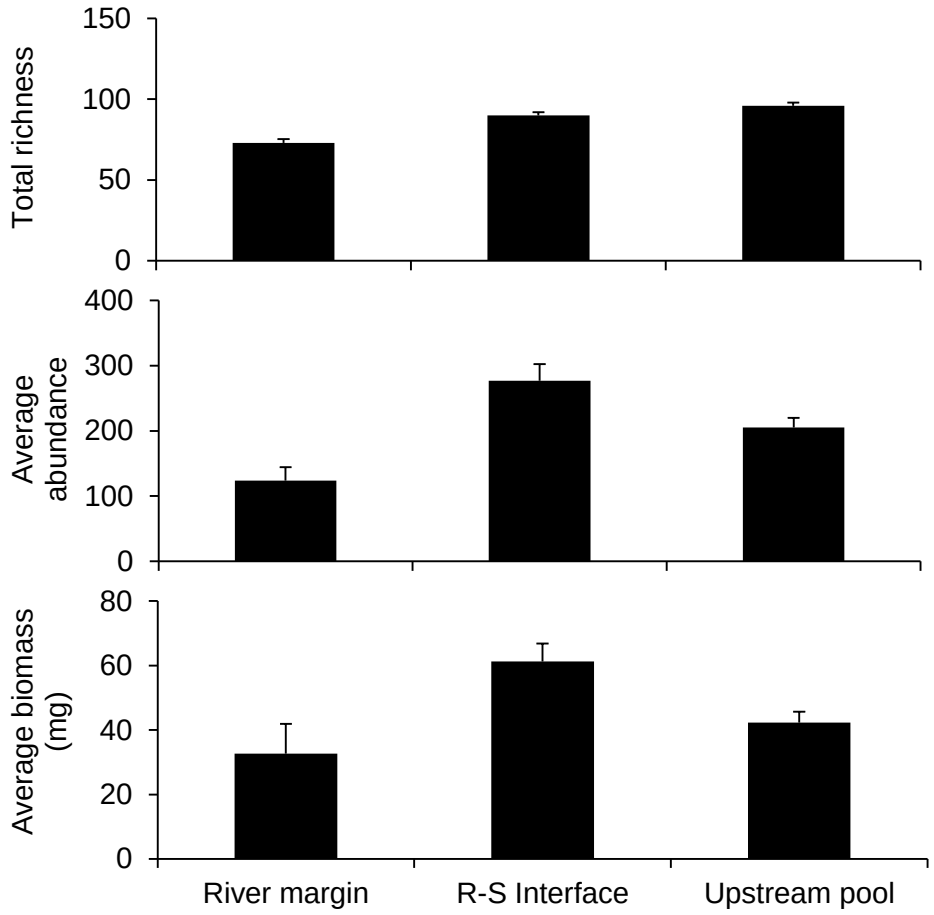


Figure 6.5. *Bar plots with richness, average abundance and biomass (AFDM) of macroinvertebrates per kick sample at the river margin, river-stream interface and upstream pool. Error bars represent standard deviation (river margin n= 11, river-stream interface n = 8, upstream pool n = 11).*

The highest abundance of shrimp were found at confluence zone 6 (upstream pool) with 58 shrimp present and a biomass of 16,314 mg (Fig 6.6). Although there was no difference in shrimp biomass between the river margin, river-stream interface and upstream pool ($F_{2, 27}$=2.3, p=0.12), the ANOVA coefficients suggest that the upstream pool had significantly higher biomass of (p=0.04). There was no difference in shrimp abundance between the three sampling sites ($F_{2, 27}$=0.91, p=0.42).

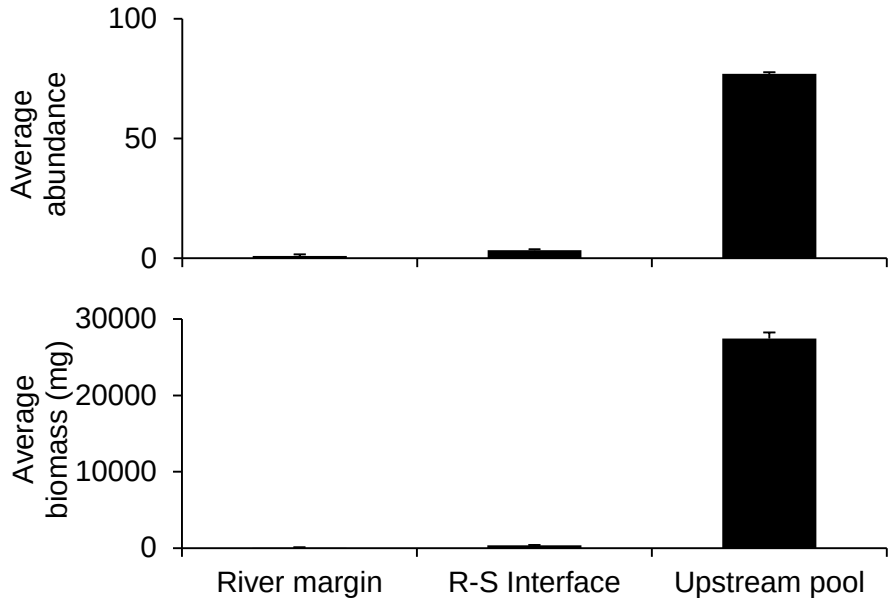


Figure 6.6. *Bar plots with average abundance and biomass (AFDM) of shrimp per kick sample at the river margin, river-stream interface and upstream pool. Error bars represent standard deviation (river margin n= 11, river-stream interface n = 8, upstream pool n = 11).*

Ephemeroptera was the most abundant Order making up 42% of individual taxa at the river margin, 28% at the river-stream interface and 29% at the upstream pool. Other abundant Orders (>10%) at the pools include Diptera, Coleoptera and Trichoptera. In terms of biomass, Ephemeroptera was also the most abundant at the river margin (51 %), river-stream interface (32%) and upstream pool (34%). However, at the river margin this was followed by Diptera (22%) and Coleoptera (18%); animals adapted to withstanding fast flows. While at the river-stream interface and upstream pool, it was followed by Odonata (26% and 17% respectively), with Trichoptera the third most abundant at the river-stream interface (12%) and Coleoptera at the upstream pool (17%). The macroinvertebrate community at confluence zone 1's river-stream interface had a structure unique compared to the other confluences, containing taxa that are normally found in fast flowing biotopes including Hydropsychidae, Eulichas (Family: Eulichadidae) and Simuliidae.

In terms of community structure, the NMDS analysis had a stress of >0.2 for both biomass and abundance data, which suggests that there were no strong differences among either the study units (e.g., R, I, U) or the eleven confluence zones (Fig. 6.1). The cluster analysis further supports this with no distinct grouping by study units or confluence zone (Fig 6.7), except the sites with shrimp as their biomass is significantly larger than other macroinvertebrates. This results in a 90% difference between the sites that contain shrimp compared with those that contain none. The ANOSIM results (Global R value = 0.09;

p=0.08) highlight the NMDS and cluster analysis findings and further demonstrate that there is little difference in macroinvertebrate communities between the river margin, river-stream interface and upstream pool. SIMPER analysis showed no site with higher average similarity above 30% (Table. 6.4). Elmidae larvae (morphospecies: genus 1) has the highest contribution of similarity to the river margin, whilst Macrobrachium (shrimp) had the highest similarity for confluence (74%) and tributary (28%). Regression analysis showed no correlation between taxa with organic matter, ambient periphyton levels or periphyton growth.

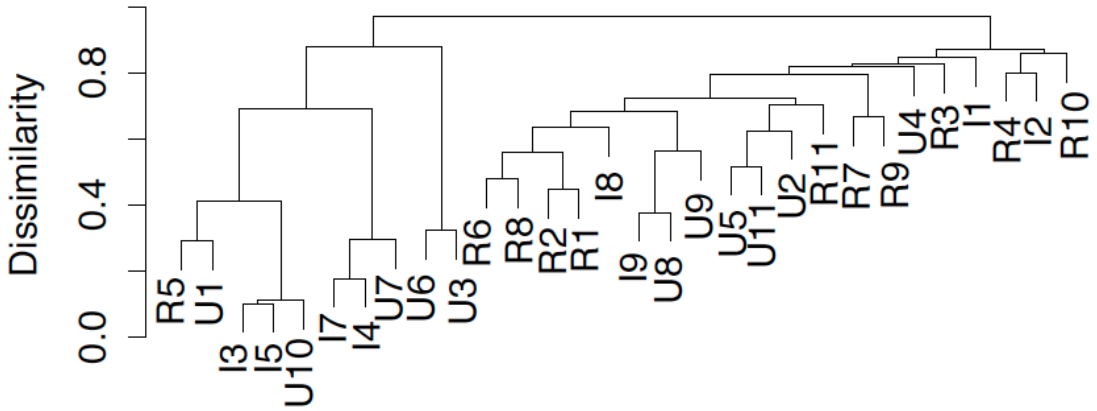


Figure 6.7. *Biomass of macroinvertebrates and shrimp with the main divide at 90+% can be explained by high abundances of shrimp. The letter represents the site; river margin (R), river-stream interface (I) and upstream pool (U), while the number is the tributary.*

Table 6.4. *SIMPER analysis of taxa contributing to the observed similarities between the biotopes. Summary results of the percentage contribution and the cumulative contribution of contributing taxa to each biotope. L= larvae*

Site & Taxa	Contribution (%)	Cumulative contribution (%)
River margin Average similarity: 25%		
Elmidae-Genus1- Larvae	29.37	29.37
Euthraulus	14.38	43.76
Caenis	13.04	56.80
Compsoneuria	7.60	64.40
Procloeon	5.80	70.21
River-stream interface: Average similarity: 26 %		
Caridea (Shrimp)	73.81	73.81
Caenis	4.48	78.29
Compsoneuria	4.00	82.29
Upstream pool: Average similarity: 28%		
Caridea	61.88	61.88
Euphaeidae	7.25	69.12
Elmidae-Genus1- Larvae	5.55	74.68

6.4 Discussion

River confluence zone ecology has predominantly focused on the influence of tributaries on the main river's ecology and geomorphology, with less work on the opposite effect of the main river affecting its tributaries (however see Beckmann *et al.,* 2005 and Wilson and McTammany 2014). The larger size of Belalong and Temburong Rivers, compared to the smaller tributaries in the catchment, means during flood events the water in these rivers have higher discharges with more potential energy. As the banks are steep with no floodplain the river is unable to expand laterally except for flowing up into their tributaries. During the frequent storm events, hydraulic damming and even reversing flows occur. The frequent and localised flood events in Ulu Temburong national park drive the interactions between the main river and its tributaries. This investigation of 11 confluence zones found the river-stream interface had higher macroinvertebrate abundance and biomass, while shrimp biodiversity is suggested to be highest in the upstream pool. However, this study found no statistical differences in organic matter or periphyton between the sites at the confluence zone.

In this study, there was no difference in the amount of organic matter (leaves, wood debris, and other material) among the confluence zone study sites. Observations of backwater channels in South America have found that close to the upstream limit of the backwater effect there is deposition of the coarser organic and non-organic matter, while closer to the river margin the finer sediments and organic matter settle out (Rosales *et al.*, 2007). In Ulu Temburong National Park there are ~250 species of trees (Cranbrook and Edwards, 1994); the tributaries that wind through the forest collect a vast array of leaf litter and wood debris, therefore the confluence has a diverse mix of organic material. The predictable pattern of flood events, which cause this hydraulic damming in the tributaries of Ulu Temburong, cause patches of organic matter to be consistently reshaped and replaced by new material being washed down from upstream. Regardless, the pattern does not seem to affect the benthic invertebrates ($R=0.01$). However, further studies are required to quantify the import and export of organic matter to understand retention times at the river confluences and assess if over longer time periods there are differences in organic matter at the confluence compared to upstream of the tributary.

The phenomenon of hydraulic damming does not just happen on the small tributaries, with interactions occurring on Temburong and its tributary, Belalong. Hydraulic damming can occur on Belalong on a weekly basis, while flood events that cause Temburong

to flow up Belalong occurring every few years (Chua, Kuala Belalong FSC manager, personal communication; Fig. 3.7). The steep valleys of the national park restrict bankful widths on the Temburong, therefore increasing the discharge, and when the river reaches the confluence of Belalong, the river either blocks Belalong's flow or it starts flowing up it. This interaction between main rivers and their tributaries can be described (and classified) as places of discontinuity, where abrupt changes in the physical and biological conditions occur. However, in another sense, tributaries and their confluences can be compared to places of lateral connectivity, likened to the interactions of rivers with their floodplains, groundwater and the sea in the form of estuaries (Ward and Stanford, 1995; Attrill and Rundle, 2002; Bunn and Arthington, 2002; Hohausová *et al.*, 2003). In catchments like Ulu Temburong where frequent flood events have the power to scour out biota and functional habitats, these tributaries could be seen as important places of storage of functional habitats and refuge for biota.

In this study, average periphyton growth rates (0.14 $g^{-2}\,d^{-1}$) and ambient periphyton levels (7.7 g m^{-2}) were similar to other streams in Ulu Temburong catchment. For example in 2014, in the pools of Sungai Lower Apan, Sungai Esu and Sungai Threelan the average periphyton growth rate was 0.06 $g^{-2}\,d^{-1}$ and average ambient periphyton was 4.1 g m^{-2}. However, confluence zone 1 river margin was an exception, with ambient periphyton levels of ~24 g m^{-2} (Table 6.3). It was expected that the river margin would have higher periphyton levels as a result of the wider channel on the main rivers, creating an open canopy and allowing more sunlight which would promote periphyton growth (Power and Dietrich, 2002; Kiffney *et al.*, 2006). However, this study found no significant difference in ambient periphyton levels or periphyton growth levels between river margin and the upstream pools. This was surprising due to the different environmental conditions, including differences in canopy cover and potential difference in other aquatic communities including fish, which are known to occur in higher abundances in the main river and promote periphyton growth rates (Pringle and Hamazaki, 1997; Moulton *et al.,* 2010). However, there are many factors (e.g., light, nutrients, grazing pressure, scour) that affect periphyton growth (Biggs *et al.*, 1998). These need to be investigated at each site to understand which are important in influencing periphyton growth in the confluence zone.

In this study a total of 123 macroinvertebrate taxa were collected, this total richness was higher compared to the richness of pools in the study tributaries of Temburong and Belalong, which are not affected by confluence disturbance (Chapter 7). In the 5 sampled

pools of Sungai Lower Apan a total of 37 taxa were reported, while 50 taxa were reported in 5 sampled pools of Sungai Esu and 61 taxa were found in the 6 pools of Sungai Threelan. Higher macroinvertebrate richness in the confluence zones compared to the pools upstream in the tributaries may be a reflection of the more dynamic hydrology. In terms of community analysis, apart from sites with shrimp present, the macroinvertebrate communities were similar in and among sites at the confluence zone (Fig 6.7). However, more effective shrimp surveying techniques are required such as trapping to get a better estimate (Jacobsen *et al.,* 2008). In other confluence studies there have been reports of similarities in macroinvertebrate communities in the main river and the tributary, which was suggested to be due to similarities in bed material between the main river and tributary, and due to adult dispersal between the tributary and main river (Wilson and McTammany, 2014). Upstream larval dispersal over short distances can also occur during base flow (Elliott, 2003) and dispersal during flood spates has been recorded (Beckmann *et al.*, 2005). The frequent floods at Ulu Temburong would suggest that the latter could occur regularly in my study sites. Blanco-Belmonte (2006) found that benthic invertebrate communities changed along the confluence zone of a backwater channel with specialist species occurring in high abundances in the more dynamic parts of the confluence. However, Blanco-Belmonte (2006) also found some taxa were not influenced by the confluence and instead by the presence of functional habitats such as wood debris, leaf litter or alluvial sediments. In my study I found no correlation between macroinvertebrates with differing quantities of organic matter or periphyton which suggests that the system is physically controlled by the frequent flood events.

Although macroinvertebrate community structure was generally similar between the sites, the SIMPER analysis (Table 6.4) did show that elmids contributed to community similarities between the river margin sites. These animals are small in size and adapt at withstanding fast flows by attaching to moss or taking refuge in between cobbles. Mayflies were also abundant in the river margin, including the flattened mayfly (*Compsoneuria)* adapted to graze algae in fast flows due to its dorsally compressed body. The community at the confluence and tributary were different to the river margin due to higher abundances of shrimp. In terms of macroinvertebrate abundance and biomass, the results suggest higher levels at the confluence site compared to the river margin and tributary (Fig 6.5). This could be due to lower disturbance compared to the river margin, being slightly protected from scouring flows at the river margin but more dynamic than tributary pools with pulses of

organic matter from the main river. Beckmann *et al* (2005) found during flood events, current velocity upstream from the tributary mouth was 40-50 % lower compared to the main channel of the Rhine. This high level of disturbance at the confluence mouth was suggested to cause lower levels of macroinvertebrates richness and total density compared with sites further upstream (Beckmann *et al.*, 2005). The lower levels of velocity just upstream from the river margin would likely to increase sedimentation and increase levels of fine substrate on the riverbed. Macroinvertebrate communities are known to be influenced by substrate (Rice *et al.*, 2001) and at my sites the highest abundance and biomass of macroinvertebrates was found with substrate comprising of ~70 % sand, which may enable burrowing to escape fast flows.

Shrimp can occur in high densities in the headwaters of tropical streams and have significant impact on ecosystem functions (Power 1984; Flecker 1992; Rosemond *et al.,* 1998). In this study two genus, *Macrobrachium* and *Caridea,* were present. The former is the most abundant and common shrimp in the streams of Ulu Temburong National Park (Wowor, personal communication, 2015). *Macrobrachium* have been found to exhibit aggressive behaviour in the Neotropics, which can cause other shrimp to take shelter (Crowl and Covich, 1994; March *et al.,* 2001). In other studies of benthic community patterns in the streams of Ulu Temburong, I have only found *Macrobrachium* (Chapter 7 and 8). It is therefore interesting that in this study *Macrobrachium and Caridea* were found together (tributary 4-confluence), and possibly at other sites but identifications of shrimp to genus level was not possible. As shrimp are omnivorous it was predicted that they would be most abundant in sites with the highest amount of organic matter (river-stream interface), instead the highest abundance and biomass was in the upstream pool, the furthest away from the main river. Fish are known to use confluences as refuge during high river discharges (Lucas *et al.,* 2001*;* Brown and Hartman, 1988) and in tropical streams high densities of shrimp are usually found in the smaller tributaries above high gradient riffles or waterfalls, where there is a refuge from fish predation (See Chapter 8; Pringle *et al.,* 1993; March *et al.,* 2002; Covich *et al,.* 2009).

6.5 Conclusion

This study suggests there is a stronger link between confluence hydrology and macroinvertebrates, than with organic matter and periphyton. The flashy flows in tropical streams create a very dynamic benthic habitat, that is constantly changing and my results suggests that the taxon reflect the hydraulic differences more accurately than single

measurements of the organic matter and periphyton. This is not surprising given that the communities often tell the 'longer story' (*e.g.*, macroinvertebrates being used as indicators for water quality; Giller and Malmqvist 1999). Similar results were found in the pilot study (chapter 4), where macroinvertebrates reflected the biotopes more closely compared to the one off physical measurements. Due to the frequency of the flood events, a long term data set would be required to understand ambient periphyton levels and the lateral two-way exchange of organic matter that occurs between the tributaries and the main rivers. In the upper part of the Temburong catchment where the channels are restricted by high banks, the tributaries may be important areas of storage for organic matter during flood events and be biodiversity 'hot spots' for macroinvertebrates. The expansion of the main rivers in the confluence zone of the tributaries during flood events may have a similar function to a floodplain and thus vitally important to the river ecosystem.

.

Chapter 7: Fluvial biotopes influence macroinvertebrate biodiversity in Southeast Asian tropical streams [2]

7.1 Introduction

Human activities are increasing the urgency for investigating basic tropical stream ecology (Dolný *et al.,* 2011, Dudgeon 2015, Lewis *et al.,* 2015, Ramírez et al. 2015). This is particularly apparent in Southeast Asia, where rising world demand for palm oil is driving deforestation. In spite of this phenomenon, large areas of the Southeast Asia tropics are not being actively studied (Ramírez *et al.,* 2015). This includes Borneo, an island that is home to one of the oldest rainforests in the world. A recent study suggests that approximately 80% of Malaysian Borneo rainforests have been severely impacted by deforestation and conversion to palm oil plantations (Bryan *et al.,* 2013). This land use change and subsequent loss of aquatic biodiversity limits the ability to study the properties of natural systems.

One approach to studying tropical stream ecology is the classification and mapping of invertebrates associated with geomorphic and hydraulic conditions. This technique has provided a robust evaluation of the importance of hydraulics, sediment dynamics, and geomorphology on temperate stream habitats (Bunn and Arthington 2002, McManamay *et al.,* 2014, Villeneuve *et al.,* 2015) and is operationally referred to as "biotope theory" (Dahl 1908, Townsend and Hildrew 1994, Newson and Newson 2000). At its core, biotope theory is based on observable environmental conditions (Jowett 1993, Wadeson 1995, Padmore 1998, Newson and Newson 2000, Clifford *et al.,* 2006). As such, biotopes refer to the abiotic environment; in streams and rivers, these are typically observed as surface flow features (i.e., flow biotopes), such as riffles, pools, and waterfalls. These biotopes reflect combinations of substrate type, depth, and velocity, which ultimately influence macroinvertebrate biodiversity (Newson and Newson 2000, Parasiewicz 2007; Table 2.1).

[2] This chapter is accepted for publication in the Journal Ecosphere

Few studies conducted in the tropics have strictly employed biotopes as a sampling framework (Furtado 1969, Quentin 1973, Dudgeon 1994, Yule 1996, Ramírez *et al.,* 1998, Principe 2008). However, other studies have modified biotope theory to assess longitudinal assemblage structure of tropical rivers (Bishop 1973, Rundle *et al.,* 1993, Greathouse et al. 2005). Not surprisingly, there is still much to learn about the mechanisms by which the structure, compositions, and patterns of biotopes can affect macroinvertebrate biodiversity in the tropics (Bisson *et al.,* 1982, Ramírez and Pringle 1998, Cheshire *et al.,* 2005, Md Rawi *et al.,* 2014).

The configuration and hydraulic properties of biotopes are highly variable and depend on the process by which they were formed and position in the channel (Bisson *et al.,* 1982). Many tropical headwaters experience flash floods and are categorized as relatively unpredictable systems (Boulton *et al.,* 2008). This range in conditions can result in biotopes, especially those with mixed substrates (i.e., pools and riffles), exhibiting a continuum of conditions, which may result in two distinct environments. For example, during low flows, tropical streams are complex systems exhibiting a mix of flow biotopes (i.e., pools, riffles, and cascades) and functional habitats (i.e., wood debris, leaf litter, cobbles, and gravel; *sensu* Harper *et al.,* 1995, Harvey *et al.,* 2008); however, during a flood event these streams become homogeneous as water rises to form a uniform a flood biotope. For naturally disturbed systems, fixed habitat features create refuge space for macroinvertebrates during high flows (Bond and Downes 2000), suggesting that some biotopes and habitat features may have a disproportionate importance on the maintenance of biodiversity (Buendia *et al.,* 2014).

It is vitally important to increase our understanding of tropical stream ecosystems in order to assess and mitigate the impacts of forest modification and destruction on biodiversity (Dolný *et al.,* 2011). Streams flowing through Ulu Temburong National Park in northern Borneo are still surrounded by unlogged primary rainforest, with no roads (Sheldon 2011). This provides a unique opportunity to study the importance of biotopes in preserving macroinvertebrate biodiversity. This paper aims to evaluate macroinvertebrate biodiversity and community structure among three study streams in four types of biotopes: pools, riffles, cascades, and waterfalls. This study specifically evaluates the importance of biotopes, rather than streams or reaches, for the operational scale of biodiversity.

7.2 Methods

7.2.1 Study Sites

This project was conducted in Ulu Temburong National Park in the Temburong district of Brunei, northern Borneo (Fig 7.1). The national park has sharp topography; the elevation of Kuala Belalong is 30 m.a.s.l., but rises to mountain peaks of 1,850 m.a.s.l. at Bukit Pagon and 913 m.a.s.l. at Bukit Belalong (Dykes 1994). The area is composed of deep V-shaped valleys with no floodplains and many waterfalls occur along the tributaries that drain the mountains. The geology is characterized by sedimentary rocks with some sandstone pebbles that have been transported from the headwaters in the southeast. Brunei has a tropical climate, which is weakly influenced by the South East Asia monsoon season (Dykes 1996). Despite the annual climate pattern, daily weather in the Temburong National Park is very erratic. Most rain originates as convection cells; as the cells rise over Bukit Belalong and Bukit Pagon, they condense, producing heavy rainfall (Cranbrook and Edwards 1994). Dykes (1997) has argued that no month can be considered dry as every month of the year receives an average of over 200 mm of rainfall.

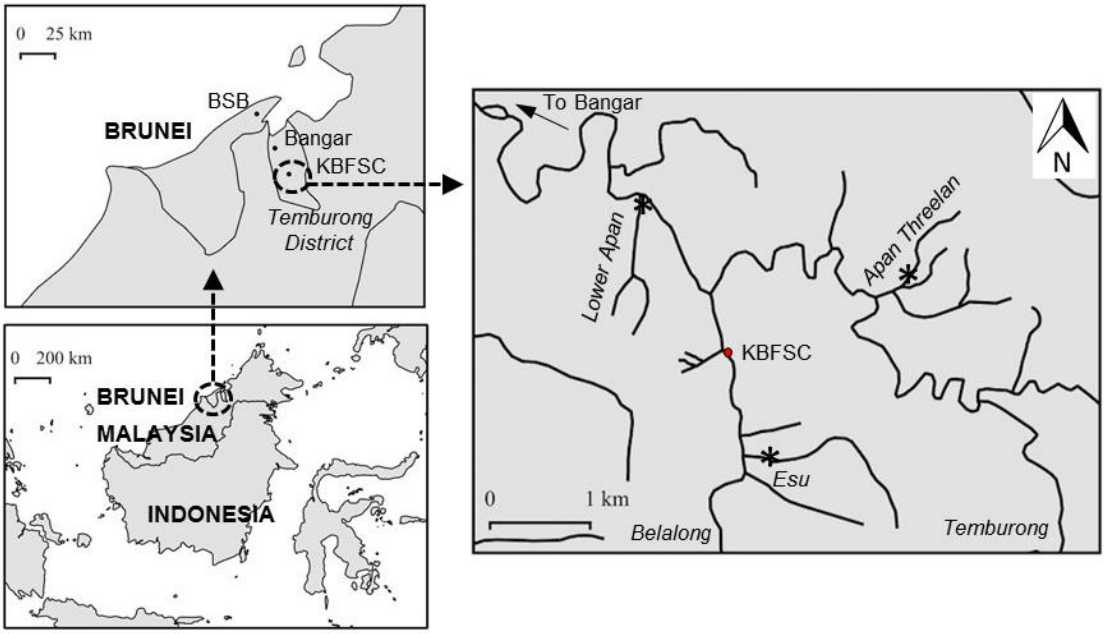


Figure 7.1. *Brunei is situated in the north of Borneo. The country is split into two contiguous regions, with Ulu Temburong National park located within the Temburong district. Kuala Belalong Field Study Centre (KBFSC) and the study reaches including Lower Apan, Esu and Threelan (highlighted by asterisks) are all within the national park.*

Three streams situated near the Kuala Belalong Field Study Centre (KBFSC) were the focus of this study: Sungai Lower Apan, Sungai Esu, and Sungai Apan Threelan (Fig 7.1). All three streams are tributaries of Sungai Belalong or Sungai Temburong and were chosen

because they each contain a mixture of biotopes. Further, these streams are uninfluenced by anthropogenic factors, and their natural water quality is high (Sheldon 2011), which is important because variation in water quality impacts biodiversity patterns (Everaert *et al.,* 2015). Lower Apan has the longest reach (90 m), exceeding those of Esu (70 m) and Apan Threelan (75 m). For each stream, the survey locations started just before the first waterfall upstream from each confluence with the main rivers (Belalong or Temburong). Esu and Apan Threelan had waterfalls higher (approximately 6 m high) than those of Lower Apan (approximately 3 m high). Sampling locations began 360 m upstream from the confluence of Apan Threelan and Temburong, 157 m upstream from the confluence of Esu and Belalong, and 60 m upstream from the confluence of Lower Apan and Temburong (Fig 7.1). Sampling was conducted during April 2013.

7.2.2 Field Methods

Biotopes (i.e., pools, riffles, cascades, and waterfalls) were mapped in each of the study reaches by observing river surface features at baseflows (Newson and Newson 2000, Parasiewicz 2007). For the Lower Apan reach, 14 biotopes were sampled: 5 pools, 2 riffles, 5 cascades, and 2 waterfalls. For the Esu reach, 10 biotopes were sampled: 5 pools, 1 riffle, 2 cascades, and 2 waterfalls. For the Threelan reach, 11 biotopes were sampled: 6 pools, 2 riffles, 1 cascade and 2 waterfalls. Across the entire study 16 pools, 5 riffles, 8 cascades, and 6 waterfalls were sampled. Features of each biotope habitat were measured. Large habitat features can form biotopes, such as boulders and tree trunks that dam the water flow (Fig 7.2).

Figure 7.2. *Extensive debris dam at a waterfall on Esu. The man indicates scale.*

Conversely, habitat features, such as leaf litter, can occur within biotopes. Physical conditions of the biotopes were measured with surveying tapes and meter sticks and included wetted and bank-full width and channel depth. Stream velocity was measured using an electromagnetic flowmeter (Valeport® model 801; Valeport Ltd., Totnes, UK). Benthic substrates were assessed visually and categorized according to percent gravel, cobble, boulder, and bedrock. The presence or absence of functional habitats was recorded including wood debris (large and small), leaf litter, and moss; trailing roots in all biotopes were also recorded.

Benthic macroinvertebrates were sampled in each biotope using a Surber sample (0.10 m^2; 250-micron mesh). Decapods are not effectively sampled by Surber sampling (Jacobsen *et al.,* 2008) and therefore were not included in this study. Due to the low macroinvertebrate densities, three samples were composited for each biotope.

7.2.3 Laboratory Methods

Owing to requirements of specimen export permits, macroinvertebrate samples were processed under (10×) magnification at KBFSC and preserved in 70% ethanol. Once exported to the UK, macroinvertebrates were identified to the lowest practical taxonomic level and enumerated; total body lengths were measured to the nearest 0.5 mm. The macroinvertebrate diversity of Borneo is still mostly undescribed; therefore, identifications were made using the few keys available, including Dudgeon (1999) and Yule and Yong (2004) as well as open source identification methods. Most specimens were identified to the genus level or morphotyped to a similar level. However, some taxa, such as Coleoptera and Diptera specimens, could only be identified to the family level (Manfred, *personal communication*, 2014; Yule 2004).

Taxa-specific ash-free dry mass (AFDM) was calculated using length–mass regressions (Benke *et al.,* 1999, Sabo *et al.,* 2002, McNeely *et al.,* 2007). When no taxon-specific equations were available, estimates were made using equations from taxa with similar body shapes (Ramírez and Pringle 1998). Where only dry mass (DM) estimates were available, values were converted to AFDM following Waters (1977).

7.2.4 Data Analysis

Macroinvertebrate biodiversity, richness, density, and biomass (AFDM) were quantified for all of the biotopes in each of the tributaries. Comparisons among tributaries

and biotopes were carried out via a two-way ANOVA followed by Tukey *post hoc* tests. Richness and density met the required statistical assumptions (i.e., normally distributed and homoscedastic residuals), but biomass was square-root transformed in order to minimize deviations from normality and homoscedasticity. Similarity percentage analysis (SIMPER) was used to identify the taxa that contributed most to the average dissimilarity among biotopes. Analysis of Similarities (ANOSIM; Clarke, 1993) was used to test for differences in abundance and composition of macroinvertebrates among the biotopes. The global R statistic, which ranges from -1 to +1, measures the distinctiveness of the grouping according to ANOSIM. Values close to 1 indicate high similarity among groups, 0 indicates that there is no relationship in composition among the groups, and -1 indicates samples are distinct to each group. Abundance data were used for both SIMPER and ANOSIM, and both of these tests use the Bray–Curtis index, a popular dissimilarity index for ecological data (Borcard *et al.,* 2012).

Macroinvertebrate assemblage structures were examined among biotopes using a hierarchical cluster analysis carried out using Bray–Curtis index values (Thomas *et al.,* 2011). Bray–Curtis dissimilarity matrices were calculated, summarizing the compositional dissimilarity of sites based on the density of taxa at each site. Nonmetric multi-dimensional scaling (MDS) analysis was used to test the robustness of groups defined by the cluster analysis. MDS is a flexible statistical tool with few statistical assumptions. The stress value was 0.16, which indicates a good ordination (Clarke and Warwick 2011). Environmental data were fitted to the ordination using the 'envfit' function of the vegan package in the statistical computing environment R (R Core Team 2013). The 'envfit' function uses mixed environmental data including both continuous variables and categorical data (Oksanen 2016). Only the statistically significant environmental variables (i.e., $p < 0.05$) were fitted and are independent to the MDS ordination.

7.3 Results

7.3.1 Site Description

Many biotopes in Lower Apan were unconstrained laterally, transitioning directly from the stream to the rainforest, whereas Esu and Apan Threelan were constrained by riparian bedrock, resulting in narrower bankfull widths. Thus, the Lower Apan had more trailing roots and terrestrial vegetation at the margins of the stream compared to the other study reaches. All of the study reaches exhibited evidence of landslides, and large wood

debris was often found to be lodged between waterfalls, sometimes creating dams. Many of these dams were quite large; for example, Figure 7.2 shows a large debris dam at a waterfall on Esu.

Esu had the highest baseflow discharge (0.92 m^3 s^{-1}) compared to Lower Apan (0.62 m^3 s^{-1}) and Apan Threelan (0.18 m^3 s^{-1}; Table 7.1 and Appendix Chapter 7a). However, bankfull width ($F_{2, 23}$ = 1.84, p= 0.18) and wetted width ($F_{2, 23}$ = 1.23, p= 0.30) did not differ among tributaries. Average depths differed significantly among tributaries ($F_{2, 23}$ = 15.79, p=< 0.001) with Apan Threelan having the shallowest biotopes (0.16 m), followed by Lower Apan (0.25 m) and then Esu (0.37 m). Average velocities were higher along Lower Apan (0.39 m/s^{-1}) than Esu (0.37 m/s^{-1}) and Apan Threelan (0.20 m/s^{-1}; $F_{2,23}$ = 4.66, p= <0.05).

Table 7.1. *Average physical conditions including depth, wetted and bankfull width, velocity, and discharge of the three study streams (pooled across all biotopes) and of the four biotopes (pooled across all study reaches).*

	Average depth (m)	**Wetted width (m)**	**Bankfull width (m)**	**Velocity (m sec^{-1})**	**Discharge (m^3 sec^{-1})**
Lower Apan	0.26	5.58	8.79	0.39	0.62
Esu	0.37	4.88	7.65	0.37	0.92
Threelan	0.16	3.76	6.78	0.20	0.18
Pool	0.47	5.47	7.15	-0.12	-
Riffle	0.14	3.42	11.89	0.48	-
Cascade	0.06	5.02	7.23	0.60	-
Waterfall	0.07	3.90	7.07	1.01	-

Bankfull widths differed among biotopes ($F_{3, 23}$ = 3.56, p=< 0.05) with waterfalls (7.07 m) having the lowest average values and riffles having the highest (11.89 m). Wetted widths also differed among biotopes ($F_{3, 23}$ = 33.95, p= < 0.05), with the lowest values occurring at riffles and waterfalls (<4 m) and highest values at cascades and pools (>5 m). Biotope depths differed ($F_{3, 23}$ = 55.14, p= < 0.001), with average values lowest for the waterfalls and cascades (<0.10 m) and highest for the pools (>0.40 m). There was a difference in velocity among the biotopes ($F_{3, 23}$ = 80.91, p= < 0.001), with the lowest average velocity in the pools (-0.12 m s^{-1}) and highest in the waterfalls (1.01 m s^{-1}). Pools and riffles contained a mix of gravel, cobbles, and boulders, whilst cascades and waterfalls were dominated by bedrock (<80% coverage; Fig 7.3). Pools had the highest percentage presence of functional habitats compared to the other biotopes with 88% of pools having leaf litter (Table 7.2). Cascades and waterfalls had the lowest percentage presence of functional habitats with moss occurring in the highest percentage (Table 7.2).

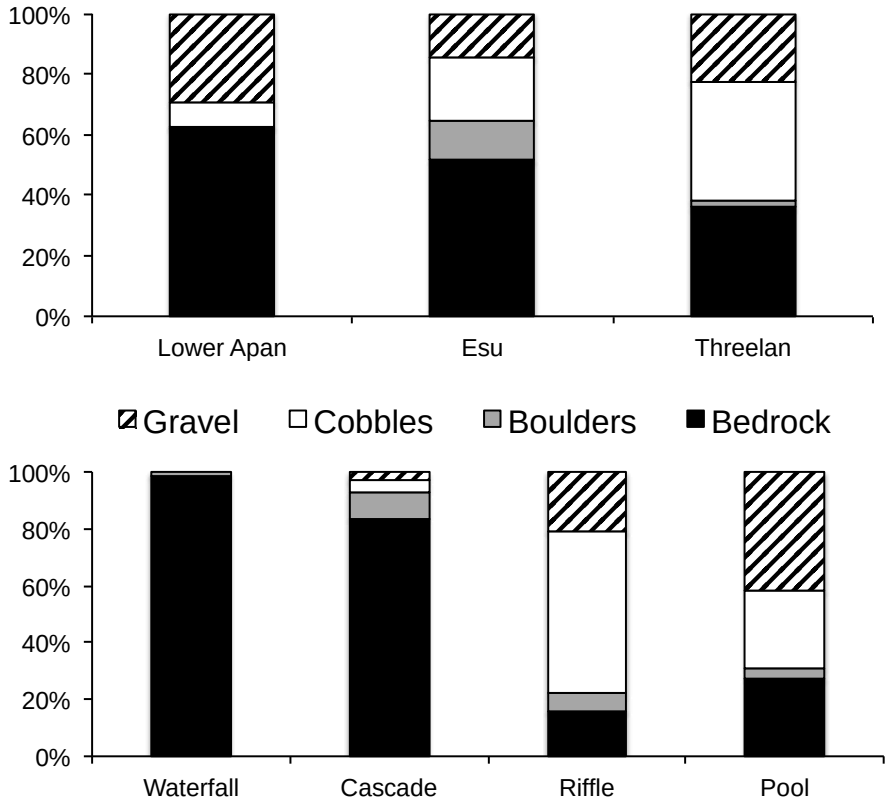


Figure 7.3. *Percentage substrate (gravel, cobbles, boulders, and bedrock) of the three study streams (pooled across all biotopes) and of the four biotopes (pooled across all study reaches).*

Table 7.2. *Percentage presence of functional habitats of the three study streams (pooled across all biotopes) and of the four biotopes (pooled across all study reaches).*

	Large wood debris %	**Small wood debris %**	**Leaf litter %**	**Moss %**	**Trailing roots %**
Lower Apan	25	38	63	44	44
Esu	44	56	50	38	6
Threelan	31	50	63	31	13
Pool	56	81	88	25	38
Riffle	19	31	31	19	13
Cascade	6	25	38	31	6
Waterfall	19	6	19	38	6

7.3.2 Biodiversity of the Study Reaches

In total, 119 taxa were collected in this study. After pooling all the biotopes in each study reach, total richness was lowest at Lower Apan (71 taxa), followed by Esu (77 taxa) and then Apan Threelan (81 taxa; $F_{3, 23} = 9.02$, $p = < 0.001$; Fig 4; Appendix Chapter 7b). Average biomass was also lowest at Lower Apan (65 mg m^{-2}), followed by Esu (176 mg m^{-2}) and then Apan Threelan (65 mg m^{-2}; $F_{3, 23} = 9.46$, $p = < 0.001$; Fig 4). There was no difference

in macroinvertebrate density among the tributaries ($F_{3, 23}$ = 2.59, p= 0.07). A Tukey *post hoc* test showed richness and biomass at Lower Apan were significantly lower than at Apan Threelan and Esu. ANOSIM showed an overall difference in the macroinvertebrate community structure among the three tributaries (global R = 0.31; p= 0.03). These differences were illustrated by the SIMPER analysis, which revealed average similarity between taxa was highest for Apan Threelan (51%), followed by Esu (47%), and then Lower Apan (41%; Table 7.3).

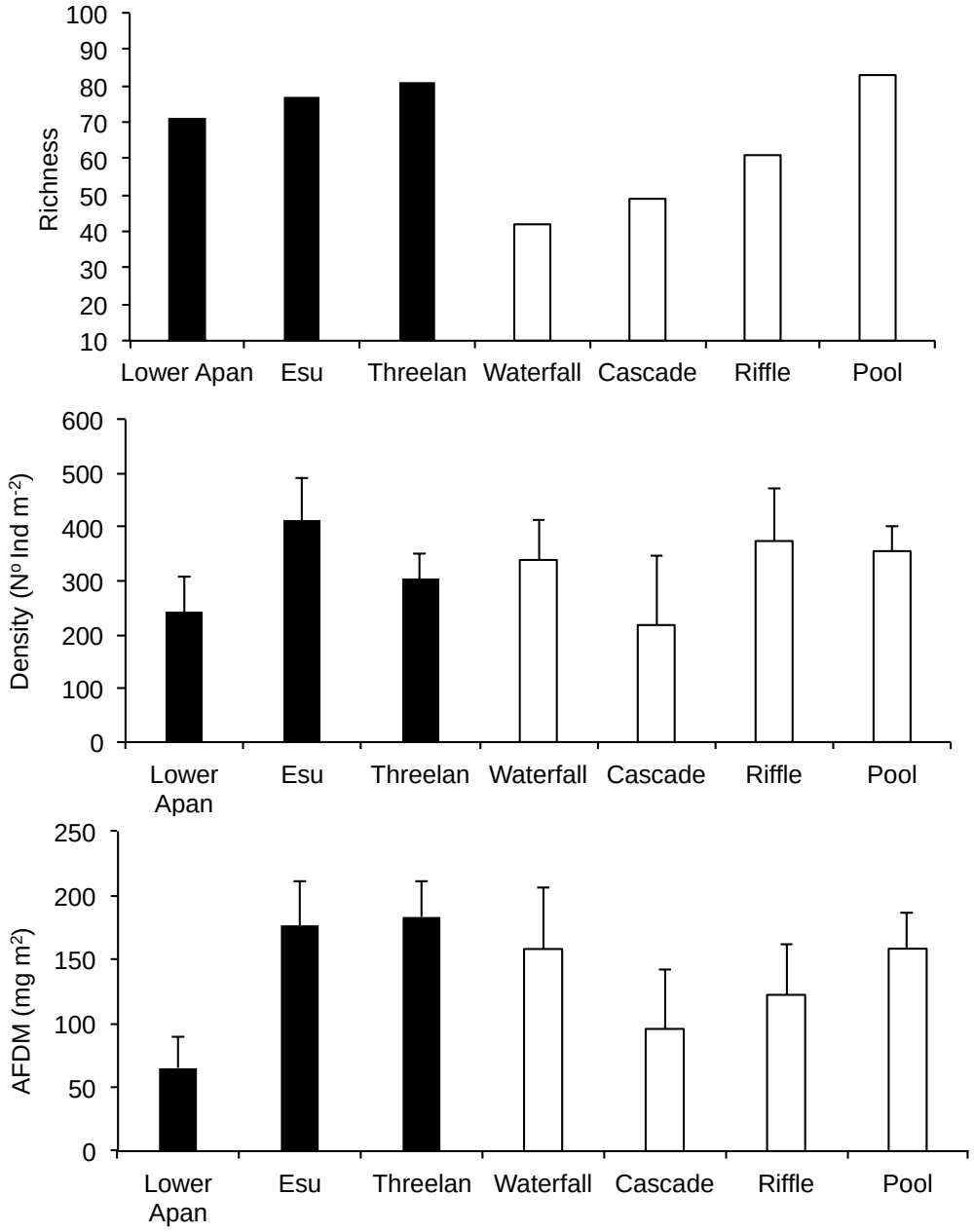


Figure. 7.4. *Richness, density, and biomass (ash-free dry mass; AFDM) of macroinvertebrates for Apan Threelan, Esu, and Lower Apan as well as for each biotope (cascade, waterfall, riffle, and pool). Error bars represent standard deviations (Lower Apan, n = 14; Esu, n = 10; Apan Threelan, n = 11; waterfall, n = 6; cascade, n = 8; riffle, n = 5; and pool, n = 16). Taxa richness ($F = 9.02$, $p < 0.001$) and biomass ($F = 9.46$, $p < 0.001$) differed among tributaries. Taxa richness differed among biotopes ($F = 3.97$, $p < 0.05$).*

Table 7.3. *SIMPER analysis of the top taxa contributing to the study sites. Percentage contribution and the cumulative contribution of contributing taxa within each biotope are shown as percentages. L= larvae*

Taxa	Percentage contribution %	Cumulative contribution %
Threelan: Average similarity: 50.59		
Elmidae Genus 1 L	13.85	13.85
Non-predacious Chironomidae	12.83	26.68
Predacious Chironomidae	12.39	39.07
Caenis	8.68	47.76
Anisocentropus	7.73	55.49
Esu: Average similarity: 46.5		
Elmidae Genus 1 L	16.76	16.76
Predacious Chironomidae	11.53	28.29
Euthraulus	9.77	38.06
Potamanthus	8.29	46.35
Caenis	7.68	54.03
Lower Apan: Average similarity: 40.96		
Simuliidae	16.65	16.65
Euthraulus	14.33	30.98
Elmidae Genus 1 L	13.84	44.83
Non-predacious Chironomidae	11.69	56.52
Neoperla	6.92	63.44

Pooling together all benthic macroinvertebrates revealed Diptera (38%) was the most abundant order, with the highest number of individuals sampled from the three streams. Other dominant orders included Coleoptera (21%), Ephemeroptera (20%), Trichoptera (9%), and Plecoptera (5%; Appendix Chapter 7b). In addition to these biodiversity measurements, there were some first recordings of aquatic insects: *Compsoneuriella* sp. (Ephemeroptera: Baetidae); *Pelthydrus elongatulus* (Coleoptera: Hydrophilidae; Schonmann 1995), *Schinostethus* sp. (Coleoptera: Psephenidae), *Dryopomorphus memei* (Coleoptera: Elmidae; Čiampor *et al.,* 2012, Sartori and Gattolliat, *personal communication*, 2014, Manfred, *personal communication*, 2014).

7.3.3 Biodiversity among Biotopes

There were differences in average richness among all biotopes (waterfalls, cascades, riffles, and pools; $F_{3, 23} = 3.97$, $p = < 0.05$). *Post hoc* tests revealed significant differences both between riffles and cascades ($p = < 0.05$) and between riffles and waterfalls ($p = < 0.05$). Based on two-way ANOVAs (using stream and biotope as factors), waterfalls at Lower Apan had

significantly lower richness than waterfalls at Esu and Apan Threelan ($F_{3, 23}$ = 3.97, p=< 0.05). However, no difference in density ($F_{3, 23}$= 0.50, p= 0.69) or biomass ($F_{3, 23}$ = 2.54, p = 0.08) was observed among the other biotopes. For overall community structure, ANOSIM showed a strong difference among biotopes (global R = 0.71; p= 0.01). This result was supported by the SIMPER analysis, which showed average similarity between macroinvertebrates was highest in the pools (51%), followed by riffles (43%), waterfalls (44%), and cascades (19%; Table 7.4). Community ordination analysis using individual taxon densities showed community structure among the biotopes (Fig 7.5; stress = 0.16; Clarke and Warwick 2001).

Table 7.4. *SIMPER analysis of the top taxa contributing to the observed similarities between the biotopes. The percentage contribution and the cumulative contribution of contributing taxa within each biotope are shown as percentages. L= larvae*

Taxa	Percentage Contribution %	Cumulative contribution %
Pool Average similarity: 51.44		
Elmidae Genus 1 L	15.95	15.95
Predacious Chironomidae	11.68	27.63
Euthraulus	11.46	39.09
Non-predacious Chironomidae	10.71	49.8
Caenis	7.70	57.51
Riffle Average similarity: 42.54		
Elmidae Genus 1 L	15.50	15.5
Simuliidae	13.51	29.02
Scirtidae Genus 1 L	9.52	38.53
Neotelmatoscopus	8.41	46.95
Neoperla	7.95	54.9
Cascade Average similarity: 19.06		
Simuliidae	28.91	28.91
Non-predacious Chironomidae	12.88 4	1.79
Hydromanicus	11.15	52.94
Neotelmatoscopus	10.66	63.6
Asiobaetodes	9.87	73.47
Waterfall Average similarity: 44.43		
Simuliidae	43.62	43.62
Neotelmatoscopus	9.47	53.08
Potamyia	9.06	62.14
Elmidae Genus 1 L	7.08	69.22
Non-predacious Chironomidae	5.51	74.73

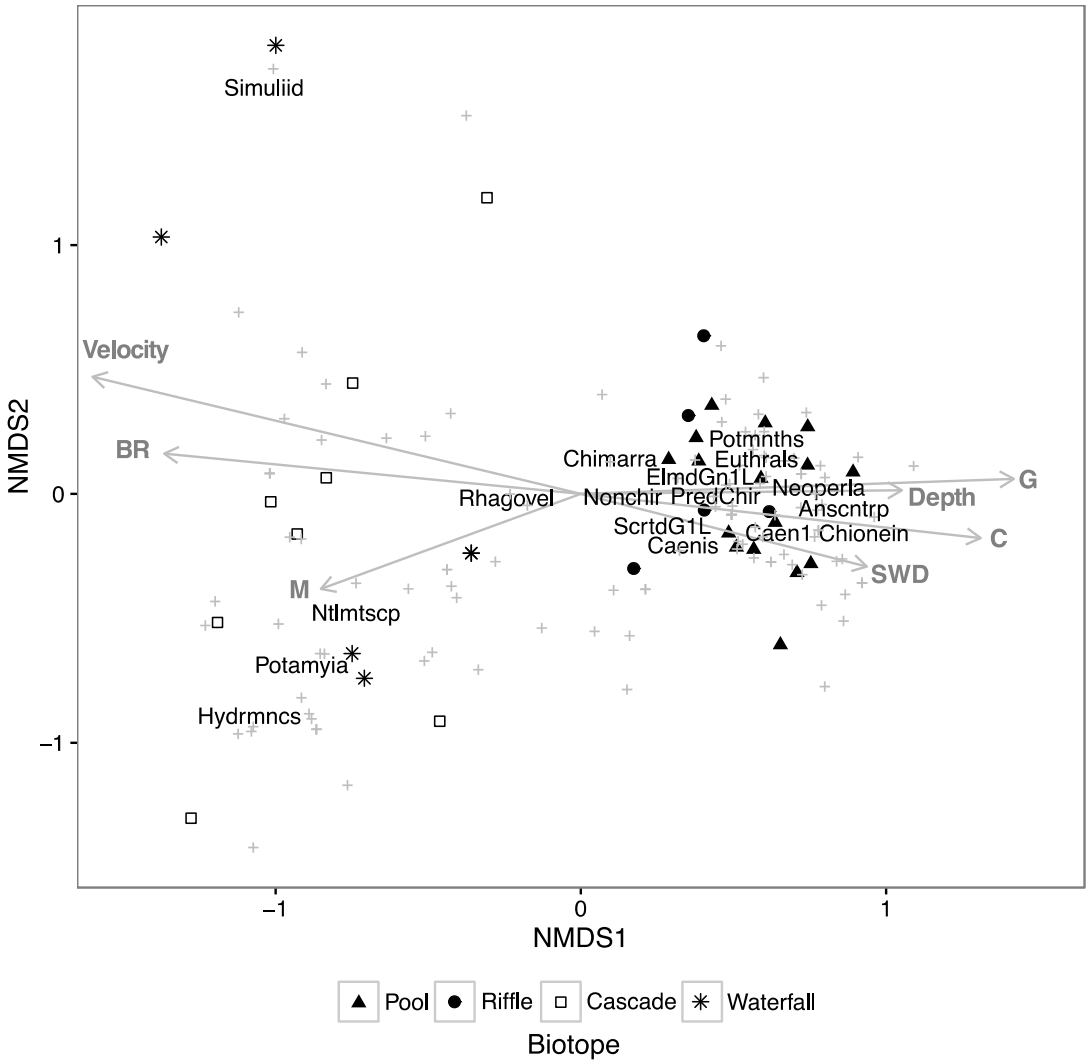


Figure 7.5. *Ordination of macroinvertebrate density among sampled biotopes. Environmental data were fitted to the ordination axis using the envfit function of the vegan package in R. The environmental factors displayed are those that are most strongly correlated with the taxa. This includes velocity, gravel (G), cobbles (C), and bedrock (BR; $p < 0.001$) as well as depth, small wood debris (SWD), and moss (M; $p < 0.05$). The biotopes are represented as symbols: pool, black triangle; riffle, black circle; cascade, white square; and waterfall, black star. List of taxa abbreviations are in the Appendix Chapter 7b (Stress = 0.16)*

Ordination axis 1 likely represented a gradient of both substrate and velocity, in which higher velocities and increased bedrock substrate were associated with waterfalls and cascades. In addition to gradients in velocity and substrate, axis 2 strongly reflected patterns in taxa richness. Specifically, sites AWF1, AWF2, and AC3 (each with <6 taxa) all clustered at the top of the plot. According to the analysis performed with BIOENV, the environmental factors that were most strongly correlated with biological variables were velocity, gravel, cobbles, and bedrock (p= < 0.001), along with depth, small wood debris, and moss (p= < 0.05). As expected, pools and riffles were associated with increased depths and areas of

deposition, with a strong association with small wood debris, gravel, and cobbles. Bedrock and high flow velocities were associated with waterfalls and cascades.

The dendrogram supports the results of the ordination analysis. There was a Bray–Curtis dissimilarity of >0.8 between the rock biotopes (cascades and waterfalls) and mixed substrate (riffles and pools; Fig 7.6). However, two waterfalls (TWF2 and EWF2) were separated from the rock biotopes, which may be explained by the higher richness of these waterfalls (25 individuals) compared to other rock biotopes.

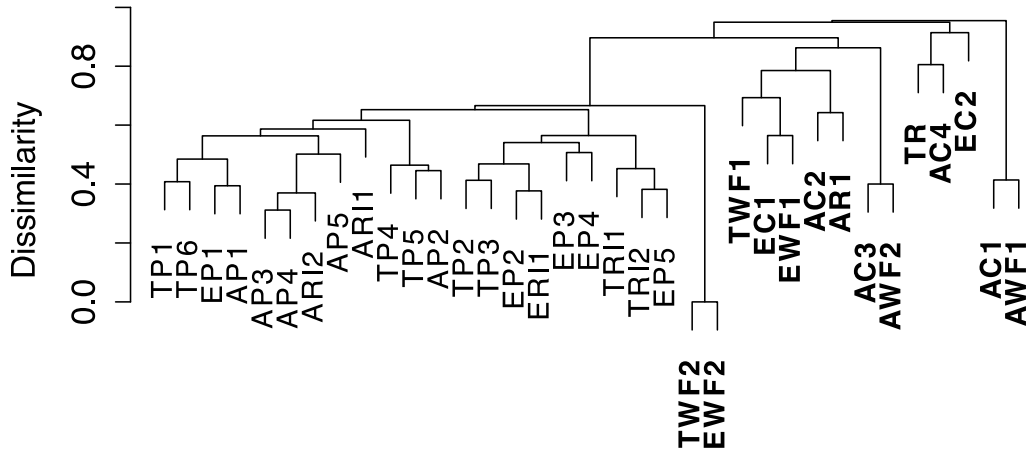


Figure 7.6. *Dendrogram of the macroinvertebrate density of the biotopes. The first letters represent the study site and biotope type: E, Esu; T, Threelan; A, Lower Apan; C, cascade; RI, riffle; W, waterfall; and P, pool. Bold text is used to highlight fast flowing biotopes (i.e., cascades and waterfalls).*

7.4 Discussion

This paper represents the first systematic study of aquatic macroinvertebrate community biodiversity in Ulu Temburong National Park; previous studies have focused on single orders, such as shrimp (Wowor and Choy 2001), dragonflies (Orr 2001), and Hemiptera (Zettle *et al.,* 2008). Additionally, very few studies have evaluated the aquatic macroinvertebrate biodiversity of the few remaining pristine catchments in Borneo, although the Sungai Wain Protected Forest in East Kalimantan (Dolný *et al.,* 2011) and Kubah National Park in Sarawak (Iwata *et al.,* 2003) are exceptions. Although these national parks protect extensive pristine rainforest, this is not the norm in Borneo or Southeast Asia (White and Klum 2008, Miettinen *et al.,* 2011). Widespread land-use degradation associated with conversion to agriculture limits the potential for baseline research and creates urgent pressure to characterize the tropical stream biodiversity of Southeast Asia. My results indicated that classification and mapping of macroinvertebrates based on biotope theory is a highly useful framework to investigate the biodiversity and community structure of tropical streams.

Furthermore, these results provide a more robust understanding of biotopes by considering the many linkages between the ecological, geomorphological, and hydrological properties that drive eco-hydromorphic complexity.

7.4.1 Biodiversity of the Study Reaches

My study reaches had higher total taxa richness (Lower Apan - 71 taxa, Esu -77 taxa, and Apan Threelan - 81 taxa) compared to similarly sized tropical streams (e.g., Sabalo stream, Costa Rica with 53 taxa: Ramírez and Pringle 1998; Rio Camuri Grande stream, Venezuela with 52 taxa: Cressa 1998). However, higher levels of richness have also been observed in Sungai Gombak (Peninsular Malaysia), where 204 morphospecies were identified (Bishop 1973); in Yuccabine Creek (northern Australia), where 267 morphospecies were recorded (Pearson *et al.,* 1986); and in a mountainous stream (Papua New Guinea), where 182 morphospecies were sampled (Yule and Pearson 1996). Dudgeon (1988) even recorded 94 morphospecies from one riffle during one day of sampling in Tai Po Kau Forest stream (Hong Kong). These differences in taxa richness among tropical streams could be caused by differences in sampling, study intensity, and duration. However, Jacobsen *et al.,* (2008) argues that these differences in taxon richness may not solely be the result of differences in sampling design and instead be due to natural regional and inter-regional patterns. Two separate studies, one conducted at a regional scale (Ecuador, Bojsen and Jacobsen 2003) and the other at an inter-regional scale (Hong Kong and New Guinea, Dudgeon 1994), had substantial differences in taxon richness between streams despite having similar sampling design.

Variation in taxon richness was found among my study streams, with Lower Apan having the lowest richness and biomass compared to Esu and Apan Threelan (Fig 7.4). Given the close proximity of all of my sites, differences are likely explained by biotope distributions and other geomorphological differences. For example, Lower Apan had the highest number of cascade biotopes, which had the lowest levels of richness and therefore decreased the sampling totals for the whole reach. Thus this study, which assessed biodiversity on a biotope scale, may have allowed for a more complete consideration of biodiversity among the study streams. Previous studies, which have just focused on single biotopes, usually riffles or pools (Dudgeon 1988, Ramírez and Pringle 1998) likely are under representing aquatic biodiversity.

Differences in biotope stream richness can also be related to the wider stream network. For example, Lower Apan was nearer to the confluence of the main river (Sungai. Belalong) relative to the other study sites. This could be associated with increased hydraulic

disturbance of the Lower Apan study reach, with scouring flows and increased interaction between the main river and the tributary, which reduce macroinvertebrate biodiversity. The proximity to the confluence also places it close to a source of fish, which are known to use the tributaries as refuges during high river discharges (Lucas *et al.,* 2001). Higher numbers of fish were present at Lower Apan compared to the other tributaries (Baker *et al.,* personal data); therefore, there may have been higher levels of predation or competition for resources. These patterns reflect processes that are affecting biotopes but are not related to stream flows.

7.4.2 Biotope Biodiversity

Differences in richness and community structure among the biotopes were most distinct between the bedrock-based biotopes (cascades and waterfalls) and mixed-substrate biotopes (pools and riffles). As shown by other studies (e.g., Rabeni and Minshall 1977, Erman and Erman 1984, Pearson 2014), current velocity, substrate size, and leaf litter were the main environmental correlates of taxon richness and community structure. Cascades consistently had the lowest richness, density, and biomass (Fig 7.4), and these were the least complex biotope, having few functional habitats. In addition, the substrate was smooth bedrock with little space for macroinvertebrate refuge (e.g., holes or crevices). In contrast, the riffles and pools had complex substrates composed of a mix of cobbles, boulders, and gravel as well as many functional habitats which support a range of macroinvertebrates (e.g., leaf litter, small wood debris, etc.), and promote higher macroinvertebrate richness. The level of waterfall complexity also appeared to affect biodiversity; some waterfalls were very simple, being composed mainly of smooth rock and thus were only habitable for filter feeders such as Simuliidae (Lower Apan, waterfall one, AWF1). Other waterfalls were geomorphically complex, with many ridges and holes, enabling a mix of leaf litter and small wood debris to become caught and thus increasing possible habitats for animals and therefore boosting biodiversity (Apan Threelan, waterfall one, TWF1). Beisel *et al.,* (2000) found the highest levels of macroinvertebrate richness occurred in heterogeneous environments that provide a range of habitats for a variety of invertebrates.

The similar macroinvertebrate communities found in pool and riffle biotopes may be attributed to frequent, high stream flow events, which occur in Ulu Temburong National Park (Dykes 1997). During these events, both biotopes experience similar hydrologic scouring events, which may have selected taxa that can withstand or require these conditions.

For example, the abundance of rheophilic Simuliidae in pool biotopes reveals the importance of such flows, as these taxa require fast flows to deliver food (Giller and Malmqvist 1999). Evidence of scouring potential in these systems was clearly demonstrated at Esu, where substrates composed of thick layers of imbricated small pebbles and gravel were frequently observed. These substrates visibly shifted after each storm event, and in spite of this high level of disturbance, Esu had amongst the highest levels of biodiversity. My observation of the importance of scouring flows for promoting macroinvertebrate biodiversity is also supported for tropical streams that experience both aseasonal (Md Rawi *et al.,* 2014) and seasonal floods (Dudgeon 1993, Leung and Dudgeon 2011, Pearson 2014).

In most stream ecology studies, floods and associated high flows are viewed as disturbance events, which are often described as 'resetting' macroinvertebrate communities (Power *et al.,* 1988, Resh *et al.,* 1988). However, in systems that flood frequently, such as streams that drain tropical rainforests, these events may not be disturbances. Regardless, flash floods play an important role in structuring resident aquatic communities, especially the biotopes with high shear stress (pools and riffles) that undergo consistent abrasion. Sheldon (2011) studied comparative habitat use by grazing fishes in rivers flowing through the Ulu Temburong National Park and found biotic interactions were minimal, with the system being physically controlled by the frequent flood events. This observation and my findings may support theories that predation and competition can be less important in highly disturbance-prone streams (Bishop 1973, Peckarsky *et al.,* 1990, Yang and Dudgeon 2010). However, much more work is needed to elucidate these patterns in these tropical streams.

7.4.3 Difficulties of Sampling Tropical Streams

Collecting representative samples in any natural environment is challenging, but it is especially difficult in the tropics because species diversity is high and many taxa are rare (Gotelli and Colwell 2011, Chao *et al.,* 2014, Hsieh *et al., unpublished manuscript*). In this study, I sampled more pools (16) than any other biotopes as these biotopes occurred between the faster flowing biotopes of riffles (5), cascades (8), and waterfalls (6). Sample-size-based rarefaction and extrapolation from the data show that from the 16 pools sampled most present taxa were likely sampled (a total of 83) and that by doubling the number of pools surveyed to 30, the number of sampled taxa would be predicted to increase to approximately 100 (Fig 7.7).

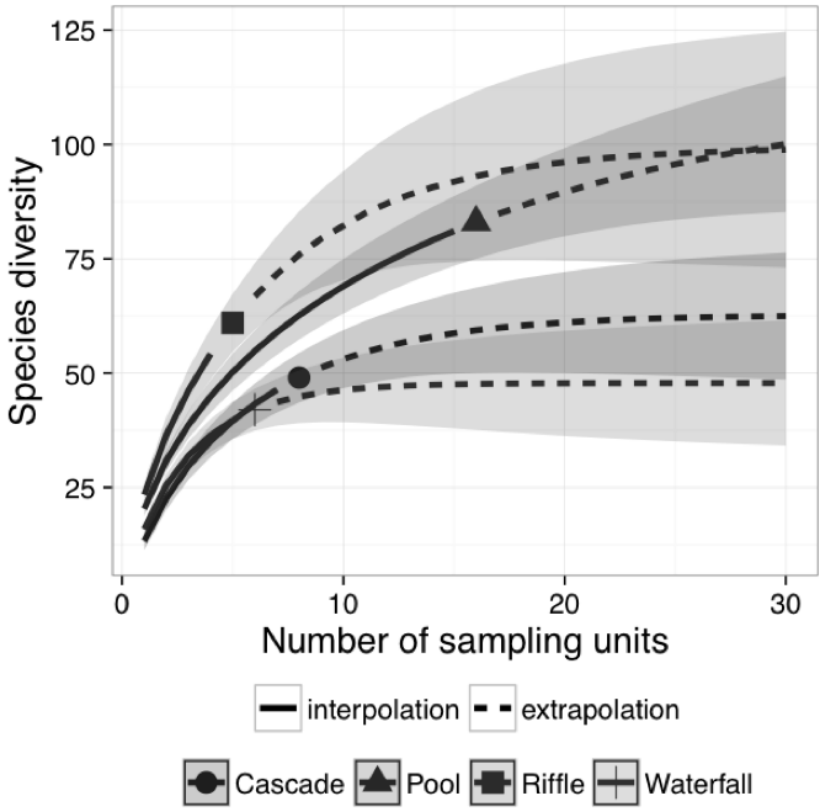


Figure 7.7. *Sample-size-based rarefaction and extrapolation showing sampling effort was sufficient for each of the biotopes: pool, n = 16; riffle, n = 5; cascade, n = 8; and waterfall, n = 6. At each of the individual biotopes, three Surber sampling replicates were taken.*

Extrapolation from riffle data shows a predicted total number of approximately 100 taxa, but this can be reached by sampling approximately 20 riffles (Fig 7.7). The taxa accumulation curve for cascades and waterfalls rise faster, showing that these biotopes have lower taxa diversity and require fewer replicates in order to sample all taxa.

These results show that my sampling efforts were sufficient to obtain an accurate representation of the benthic macroinvertebrate communities that live in the biotopes. Mixed-substrate biotopes are more difficult to sample, owing to their range of substrate and functional habitats; however, my sampling effort was sufficient to obtain a strong representation of the taxa present. In the tropics, it is common for biodiversity surveys to miss taxa because of the high number of rare taxa, thus creating a slowly rising species accumulation curve (Gotelli and Colwell 2011).

7.5 Conclusion

Recent land-use changes caused by the widespread growth of the palm oil industry in Southeast Asia have hastened the need to identify and study the remaining pristine rivers

and streams. The simplicity of biotopes, which are easily identifiable by their surface flow, combined with the reliability of macroinvertebrates as indicators of environmental health makes the approach of this research a useful one for future studies of tropical rivers and streams. This study has demonstrated that the bedrock biotopes (cascades and waterfalls) and mixed substrate biotopes (pools and riffles), harboured different communities, and levels of taxa richness. The more consistent environmental conditions of waterfalls and cascades, in comparison to the mixed-substrate biotopes (pools and riffles), appeared to have a strong influence on the macroinvertebrate communities. Macroinvertebrates inhabiting waterfalls and cascades form a community that differs from those of pools and riffles. This study shows that tropical macroinvertebrate communities are associated with biotopes rather than streams. Understanding how the different biotopes contribute to reach-scale biodiversity is therefore vitally important for the conservation of stream ecosystems.

Chapter 8: Benthic Community Structure & Ecosystem Functions in Above-and Below-Waterfall Pools in Borneo[3]

8.1 Introduction

Tropical streams often support large populations of macroconsumers, such as herbivorous fish, crabs, tadpoles and shrimp (Power, 1984; Flecker, 1992; Rosemond *et al.,* 1998), which can have a significant impact on stream ecosystems via predation and/or competition for food resources like leaf litter and periphyton among a range of other resident animals (Pringle & Hamazaki, 1997; Rosemond *et al.,* 1998). Leaf litter is often abundant and important as a functional habitat (*sensu* Harper *et al.,* 1995) in tropical headwater streams and is broken down by aquatic animals (i.e. shredders; Wootton & Oemke, 1992; Crowl *et al.,* 2001; March *et al.,* 2001; Yule et al., 2009; Coughlan *et al.,* 2010). Periphyton covers many benthic surfaces in tropical streams and is the main food source for herbivorous animals, which can limit both periphyton standing stocks and growth rates (Power, 1984; Flecker, 1992; Feminella & Hawkins, 1995; Steinman, 1996; Pringle & Hamazaki, 1997; Flecker & Taylor, 2004; Moulton *et al.,* 2015).

While availability of food resources is important for regulating both community structure and ecosystem functions, physical factors are also important (Bond & Downes, 2000). Waterfalls are fast flowing, rocky biotopes with distinct geomorphic structure (e.g. channel slopes and stream channels typically erode down to parent material). These biotopes flow vertically without obstruction and are generally more than 1 m in height (Newson & Newson, 2000; Fig 8.1). In spite of their potential importance in tropical streams, waterfalls have not received as much attention as other biotopes (Rackemann *et al.,* 2013; Clayton and Pearson, 2016). In high elevation localities, waterfalls become common because the stream gradient steepens and the river channels form discrete, sequential pools and waterfalls (or cascades and riffles). The discontinuities in channel form caused by waterfalls are barriers to the upstream dispersal of fish and thus create discrete communities (Creed, 2006; Covich *et*

[3] This chapter is accepted for publication in the Journal of Hydrobiologia

al., 2009; Hein & Crowl, 2010; Karssing *et al.,* 2012; El-Sabaawi *et al.,* 2015). In tropical streams, below-waterfall pools support a range of predacious and herbivorous fish (Choy, 1996); in contrast, above-waterfall pools tend to have few fish and often become a refuge for shrimp (Covich *et al.,* 2009; Hein & Crowl, 2010). However, Bass (2007) notes that different aquatic communities in above- and below-waterfall pools may be the result of differences in microhabitats or other environmental conditions.

Figure 8.1 *Waterfall at Sungai Esu (photo on the left) and at Lower Apan (Photo on the right)*

The different community structures of fish and shrimp above and below waterfalls can influence a range of ecosystem functions including organic matter decomposition and periphyton growth (Greathouse & Pringle, 2006; Moulton *et al.,* 2010; Ho & Dudgeon, 2016). Below waterfalls, high numbers of fish can play an important role in controlling periphyton growth by clearing rock surfaces after frequent tropical flood events (Pringle & Hamazaki, 1998). Above waterfalls, where shrimp can be abundant, organic matter breakdown rates may be increased by consumption and shredding of leaf litter by resident shrimp (Crowl *et al.,* 2001; March *et al.,* 2001; Coughlan *et al.,* 2010). Fish and shrimp can also reduce macroinvertebrate densities, as many are insectivorous (Pringle & Hamazaki, 1998). Macroinvertebrates, a term which refers to invertebrates excluding shrimp in this study, are

also important in restricting periphyton growth (Feminella & Hawkins, 1995; Steinman, 1996; Moulton *et al.,* 2015) and breaking down organic matter (Cheshire *et al.,* 2005; Masese *et al.,* 2014). Therefore, fish and shrimp may have an indirect impact on ecosystem functions by reducing macroinvertebrate densities. The separation of communities and its impact on ecosystem structure and function have been researched to some extent, with mixed results, in the Neotropics (Pringle & Hamazaki, 1998; March *et al.,* 2002; Ramirez & Hernandez-Cruz, 2004; Covich *et al.,* 2009). However, there have been few similar studies conducted in tropical Asia despite the similarity of the region's biotic and abiotic conditions to those of the Neotropics (Mantel *et al.,* 2004; Ho & Dudgeon, 2016).

The natural fragmentation of tropical streams by waterfalls provides a "natural experiment" for examining these biotic and physical factors. This study investigated four streams in Ulu Temburong National Park, Brunei Darussalam, all with waterfalls that reduce the upstream dispersal of fish, creating a refuge for shrimp in above-waterfall pools. The aim of this study was to investigate the extent to which waterfalls influence fish community structure and how expected declines in fish densities in above-waterfall pools affect the density and biomass of both shrimp and non-shrimp macroinvertebrates. In addition, I tested the hypothesis that ecosystem functions (i.e. periphyton growth and leaf litter breakdown rates) differed between above- and below-waterfall pools. I hypothesized that both shrimp and macroinvertebrates occur in higher densities in the above-waterfall pools lacking fish and that periphyton growth rates are higher in above-waterfalls pools owing to the lower number of herbivorous fish. Leaf litter breakdown rates were also hypothesised to be higher in above-waterfall pools owing to the higher numbers of shrimp and macroinvertebrates.

8.2 Methods

8.2.1 Study Sites

Small tributaries of Sungai Belalong and Sungai Temburong in the Temburong district, Brunei Darussalam (in northern Borneo) were the focus of this study (Fig 8.2). The four streams (Sunagi Lower Apan, Sunagi Baki, Sunagi Esu and Sunagi Apan Threelan; Fig 8.2) were chosen as they have perennial flows. These catchments are covered in primary rainforest, and their geology is composed of sedimentary rocks (Sheldon, 2011). The climate in Brunei Darussalam is tropical and weakly influenced by the Southeast Asia monsoon (Dykes, 1996). The daily weather pattern in the rainforest of Ulu Temburong is very erratic, with most rain originating as convection cells over the ocean. As the cells move inland they

are forced to rise over the mountains of Bukit Belalong and Bukit Pagon, condensing and producing daily heavy rain (Cranbrook & Edwards, 1994). Topography ranges from 30 m a.s.l. at the Kuala Belalong Field Study Centre but rises to 913 m a.s.l. at Bukit Belalong and to 1,850 m a.s.l. at Bukit Pagon (Dykes 1994). The streams drain V-shaped valleys with steep bank slopes. Study sites were in the vicinity of the Universiti Brunei Darussalam Field Studies Centre (FSC), which is located in Ulu Temburong National Park.

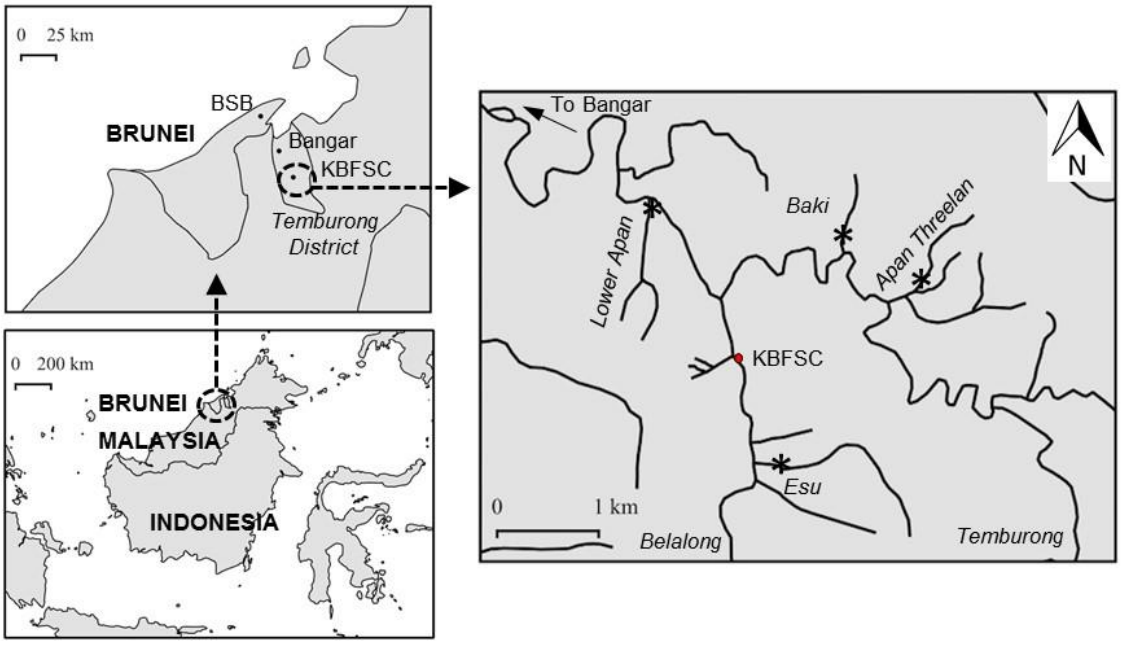


Figure 8.2. *Map showing the location of Brunei Darussalam in northern Borneo and the Temburong District. The four study streams were Sungai Lower Apan, Sungai Baki, Sungai Threelan and Sungai Esu, which are highlighted along with the main rivers Sungai Belalong and Sungai Temburong*

Water quality can have a strong impact on benthic communities and ecosystem functions (Giller & Malmqvist, 1998; Everaert *et al.,* 2014). This study was therefore conducted in streams that are not under any anthropogenic impacts in the catchment (Sheldon, 2011). Further, the similar geology and catchment condition suggest water quality should be similar among all the study streams. In pilot work, both nitrate (< 0.55 mg l^{-1}) and phosphate (< 0.08 mg l^{-1}) were found to be under the level of detection. Any variation in ecosystem structure and function in the streams of Ulu Temburong National Park are thus assumed to be attributable to natural abiotic conditions, biotic interactions among resident taxa (e.g. competition for resources and predation) and physical disturbance cause by variable river discharges.

Physical disturbances are assumed to be similar across all pool biotopes in my study streams owing to the consistent gradient of the streams. Light levels were also assumed to

be similar across my study pools, with the gap in the canopy created by the waterfalls extending to both the above-and-below waterfall pools. Lower Apan was the longest reach (90 m) of the four reaches, followed by Apan Threelan (75 m), Esu (70 m) and Baki (55 m). The waterfalls at Threelan and Baki were furthest from their respective confluences with Temburong compared to the other study streams (separated by over 300 m). This may affect the numbers of fish present in the tributaries, with fish using the tributaries as refuges during high discharges. The waterfall at Esu was approximately 150 m from its confluence with Belalong, and Lower Apan was approximately 60 m from its confluence with Temburong (Table 8.1).

Table 8.1. *Average physical conditions of the pools at the four study sites including bankfull width, wetted width, average depth, average velocity and dominant substrate type. Dominant substrate type is given in percentage cover as G, gravel; BR, bedrock; B, boulders and C, cobbles. Pool substrate compositions significantly differed between above- and below-waterfall pools (chi-squared, Lower Apan $p \leq 0.001$; Baki $p \leq 0.001$; Esu $p \leq 0.001$; Threelan $p \leq 0.001$).*

Stream	Metres from confluence (m)	Above versus below waterfall	Bankfull width (m)	Wetted width (m)	Depth (m)	Velocity (ms^{-1})	Average substrate type (%)			
							G	BR	B	C
Lower Apan	60	Below	11.55	5.07	0.29	0.51	72	2	7	13
		Above	8.68	5.46	0.76	0.19	67	32	0	1
Baki	336	Below	11.37	6.29	0.39	0.4	73	2	1	24
		Above	9.65	4.65	0.33	0.10	45	15	33	8
Esu	157	Below	6.98	4.74	0.54	0.09	20	32	20	28
		Above	7.15	7.15	0.64	0.17	27	50	0	23
Threelan	356	Below	7.08	4.55	0.24	0.08	42	2	2	58
		Above	5.6	4.45	0.28	0.01	70	40	3	33

8.2.2 Background Community Structure

A total of 44 species of fish from 10 families and 30 genera have been identified in the streams and rivers of Ulu Temburong National Park (Choy & Chin, 1994). Species of Cyprinoidea were predominant, with families Balitoridae (river loaches) and Cyprinidae being the most commonly represented (Choy and Chin, 1994). In general, cyprinids have a mixed diet that changes with their life stage and includes insects, algae, diatoms and higher plants (McConnell *et al.,* 1987), while the endemic genus Gastromyzon (Balitoridae) comprises of herbivorous fish that feed on periphyton exclusively (Tan, 2006).

Three genera of freshwater shrimp live in the streams of Ulu Temburong National Park with the most common being Macrobrachium (Decapoda: Palaemonidae; Wowor, personal communication). Macrobrachium are omnivorous, but predominantly predaceous (Crowl & Covich, 1994). The other shrimp in Ulu Temburong, which were not present in this study, include Caridina (Decapoda: Atyidae), which are found in headwater streams, and Atyopsis (Decapoda: Atyidae), which are present in streams with strong currents (Wowor, personal communication). A previous study of non-shrimp macroinvertebrates in these streams (K. Baker, unpublished data) identified 14 orders, with Diptera being the most abundant in number of individuals (approximately 40%), followed by Coleoptera (approximately 20%), Ephemeroptera (approximately 20%) and Trichoptera (9%).

8.2.3 Physical Habitat Measurements

Wetted and bankfull widths and channel depths were measured in three pools above the waterfall and three pools below the waterfall, except at Esu and Lower Apan, where there were only two pools below the confluence. At these locations, the benthic substrate was assessed visually and characterized in percent gravel, cobble, boulder and bedrock. Stream velocity was measured using an electromagnetic flowmeter (Valeport® model 801; Valeport, Totnes, UK). A two-way analysis of variance (ANOVA) was used to assess differences in the width, wetted width, depth and velocity of the study pools among streams and between the pools above and below the waterfalls. This analysis was carried out in the statistical computing environment R (R Core Team 2013).

8.2.4 Macroinvertebrate, Shrimp and Fish Sampling

Macroinvertebrates and shrimp were sampled in three pools above each waterfall and in all the pools below the waterfall to the confluence. Surber samples (0.1 m^2; 250-µm mesh) were used to collect macroinvertebrates, excluding shrimp. Three Surber samples were randomly selected in each pool where it was shallow enough to sample and composited together to create one sample. A combination of three Surber samples and a single three-minute kick sample with a standard D-frame net (500-µm mesh) was used to sample freshwater shrimp in each pool. Shrimp and macroinvertebrates were considered separately, as shrimp are significantly larger than the other macroinvertebrates and are more vagile. Samples were preserved in 70% ethanol in the field and processed under (10×) magnification in the laboratory. Individuals were identified to the lowest practical taxonomic level (most often to genus or morphotyped to an assumed similar level) and measured to the nearest 0.5

mm. Macroinvertebrate taxonomic knowledge in northern Borneo is poor; therefore, identifications were conducted using the few keys available (e.g. Dudgeon, 1999; Yule & Yong, 2004; Sangpradub *et al.,* 2006), alongside open source identification methods. Specifically, taxa were photographed and uploaded onto the Flickr website (http://flickr.com/photos/tropical-streams/sets/), where interested experts commented or requested to see the actual specimens. Ash free dry mass (AFDM) of macroinvertebrates was estimated using length–mass regressions (Benke *et al.,* 1999; Sabo *et al.,* 2002; McNeely *et al.,* 2007). In cases in which no taxon-specific equations were available, estimates were made using equations from taxa with similar body shapes (Ramírez & Pringle, 1998). Where only dry mass (DM) estimates were available, values were converted to AFDM following the calculation described by Waters (1977). All macroinvertebrates were considered to be capable of feeding on periphyton and organic matter except known predators, i.e. Odonata, Hemiptera and Hydracarina (Jinggut, 2015). Unfortunately macroinvertebrate and shrimp specimens from Baki were severely damaged in transit, so the analysis for macroinvertebrates and shrimp includes only those from Lower Apan, Esu and Threelan.

In contrast to the macroinvertebrate sampling procedures, fish were only sampled in pools directly above and below the first waterfall upstream from each confluence. Fish were sampled with a cast net (mesh size 0.64 cm^2) thrown into each of the sample pools three times. Fish were photographed and identified following the methods described by Choy & Chin (1994). Fish were categorized as herbivorous or predacious using appropriate scholarly literature, and percentages of fish belonging to these categories were calculated (McConnell *et al.,* 1987; Tan, 2006; Kottelat, 2013). Once processed, fish were immediately returned to streams.

ANOVAs were used to assess differences in biodiversity of macroinvertebrates and shrimp among study sites and between pools above and below the waterfalls, followed by Tukey's tests to identify significant factors. As fish were only sampled in pools directly above and below the waterfall, it was not possible to assess differences among study sites. For the analysis, each pool was considered as a replicate, rather than the samples within each pool. To satisfy the assumptions of normality and homoscedasticity, fish and shrimp data were transformed [using a $\log_{10}(n + 1)$ transformation]. Macroinvertebrate assemblage structure in above- and below-waterfall pools were examined via a non-metric multidimensional scaling (NMDS) analysis using abundance data followed by an analysis of similarities (ANOSIM; Clarke, 1993) to test for differences in abundance and composition of macroinvertebrates among the above- and below-waterfall pools. Surber samples were

combined to give a single estimate per pool. The statistical computing environment R (R Core Team 2013) and the R packages mass and vegan were used to conduct the ANOVA, NMDS and ANOSIM procedures. The Bray–Curtis dissimilarity index, a dissimilarity index for ecological data, was used for the NMDS procedure (Borcard *et al.,* 2012).

8.2.5 Ecosystem Function Estimates

Leaf litter decomposition rates and periphyton growth estimates were conducted in the pools directly above and below the waterfall at each study stream. The sections below describe methodologies used to estimate these ecosystems functions.

Leaf litter decomposition: Leaves from the genus *Campnosperma* (Family: Anacardiaceae) were used to estimate decomposition rates in pools above and below waterfalls. Although there are approximately 250 species of trees (Cranbrook & Edwards, 1994) in Ulu Temburong National Park, *Campnosperma* was most abundant in the study streams and therefore chosen for this experiment. Decomposition rates were estimated using standard litter bag techniques (Irons *et al.,* 1994; Benstead, 1996). Prior to the experiment, leaves were dried at 70°C for 24 hours, cooled and then weighed. Three grams of leaves were placed into nylon litter bags (mesh size, approximately 8 mm). Leaf litterbags were placed at Lower Apan, Esu, Apan Threelan and Baki. Litter bags were collected from the study sites after 7, 23, 26 or 71 days in the field and processed immediately in the KBFSC laboratory. The leaves were picked out by hand and the remaining material was processed through a 250-μm sieve, with macroinvertebrates picked out and preserved in 70% ethanol. Macroinvertebrates were then identified and measured using the methods described above. Leaf material remaining was dried at 70°C for 24 hours, cooled and weighed. Breakdown rates (k_d) were calculated using the equation

$$k_d = \frac{\ln(\text{final mass}/\text{initial mass})}{\text{days}}$$

Litter bag experiments were conducted in both 2013 and 2014. In the first year, three replicates per study pool were left for 7 and 23 day exposures. In the second year, the same methods were repeated except four replicates were used and leaf litter bags were deployed for longer periods of time, 26 and 71 days, in order to take account of the scouring effects of high discharge.

Periphyton Growth: Periphyton growth rates were estimated from the same pools that were used in the leaf decomposition experiment. Random rocks were selected from each

stream, placed in boiling water for 10 minutes and scrubbed with a wire brush to remove all periphyton. Rocks were then placed into the study pools with plastic wire used to secure them to the riverbed. After exposure, periphyton levels were estimated from rock scrubs. A fixed area (0.002 m^2) of each rock was sampled with material removed collected on GFF filters. Filters were dried at the KBFSC and transported back to the UK for AFDM analysis to be conducted. AFDM was calculated by taking the difference between the dry biofilm weight and ash weight (obtained from heating samples to 500ºC for 1 hour). AFDM was then used as an estimate of periphyton abundance. Periphyton growth rates were calculated as the difference between final and initial standing stocks (AFDM) divided by the total exposure time (days). It should be noted that our estimates of periphyton cannot distinguish between living and dead algal cells (Wellnitz & Poff, 2006). This method was selected because of the remote locations and limited facilities of the KBFSC. Periphyton experiments were conducted in both 2013 and 2014. In the first year, four rocks per study pool were left for 23-day exposures. In the second year, the same methods were repeated, but with five replicate rocks and each was deployed for a longer period of time, for 71-day exposures. In each year, ambient levels of periphyton were estimated from four random rocks in each study pool in order to ensure periphyton levels from the experiment were in line with background standing stock.

Data analysis: Separate two-way ANOVAs were used to evaluate differences in both leaf litter breakdown rates and periphyton growth rates among the study streams as well as between the above- and below-waterfall pools. In order to satisfy the assumption of normality and homoscedasticity, many of the datasets collected were transformed. A $\log_{10}$ $(n + 1)$ transformation was applied to the 7-day exposure leaf litter bag macroinvertebrate data and the 23-day exposure leaf litter bag macroinvertebrate biomass and abundance data from 2013. A square root transformation was used on the 2013 and 2014 ambient periphyton rock scrub data as well as the 2014 litter bag data on macroinvertebrates, including the 28-day exposure macroinvertebrate biomass and richness data, the 71-day exposure macroinvertebrates biomass data, the 28-day exposure non-predacious macroinvertebrate abundance data, and the 71-day exposure macroinvertebrate biomass data. Tukey's *post hoc* tests were used to identify differences among streams and between above- and below-waterfall pools.

8.3 Results

8.3.1 Physical Habitat

There were no significant statistical differences in pool widths, depths or velocities either among streams or between above- and below-waterfall pools (Table 8.1). The substrates of above-waterfall pools were 36% bedrock compared to 7% bedrock in below-waterfall pools. In contrast, there was a higher substrate percentage of cobbles in below-waterfall pools (31%) compared to above-waterfall pools (17%).

8.3.2 Macroinvertebrate, Shrimp and Fish

Total macroinvertebrate richness in the pools differed among the streams with 37 taxa at Lower Apan, 50 at Esu and 61 at Threelan (two-way ANOVA, $F_{2, 12} = 4.62$, $p = 0.04$; Appendices Chapter 8a,b&c). Average density was lower at Lower Apan (205 ± 29 individuals m^{-2}) than at Threelan (300 ± 54 individuals m^{-2}) and Esu (531 ± 98 individuals m^{-2}; $F_{2, 12} = 6.52$, $p = 0.01$). Biomass was also lower at Lower Apan (58 ± 7 mg m^{-2}) than at Threelan (187 ± 47 mg m^{-2}) and Esu (213 ± 54 mg m^{-2}; $F_{2,12} = 9.25$, $p = 0.005$). There were no differences between above- and below-waterfall pools in total macroinvertebrate richness ($F_{1, 12} = 0.47$, $p = 0.51$), density ($F_{1, 12} = 0.02$, $p = 0.89$) or biomass ($F_{1, 12} = 0.16$, $p = 0.70$). Additionally, the NMDS showed no clear pattern or difference in macroinvertebrate community structure between above- and below-waterfall pools (stress value, >0.2; Appendix Chapter 8d), and the ANOSIM procedure revealed no difference between the macroinvertebrate community structure of the above- and below-waterfall pools (global $R = 0.35$; $p = 0.24$).

Only one shrimp taxon (*Macrobrachium*) was present in the three study streams Lower Apan, Esu and Threelan (Fig 8.3 and Appendix Chapter 8e). Shrimp abundance differed among the study streams from 0.2 ± 0.13 individuals at Lower Apan to 7.1 ± 3.17 individuals at Threelan ($F_{2,12} = 4.3$, $p = 0.04$). Shrimp biomass varied from 20.7 ± 19.80 mg at Lower Apan to 2,217 ± 1,182.74 mg at Threelan ($F_{2,12} = 5.6$, $p = 0.02$). Shrimp abundance was highest in above-waterfall pools (7.67 ± 2.47 individuals versus 0.14 ± 0.10 individuals; $F_{1, 12} = 18.75$, $p = 0.001$; Fig 8.3). Shrimp biomass was also highest in above-waterfall pools (2,701.02 ± 618.31 mg versus 19.61 ± 20.93 mg; $F_{1, 12} = 24.55$, $p = 0.0005$).

It was not possible to identify all fish to the species level, particularly the smallest individuals. However, six distinct taxa were sampled across the four streams with a total of 75 individuals collected during the survey (Appendix Chapter 8f). The most abundant fish

were cyprinids, comprising 97% of observed fish, with the most abundant species being *Nematabramis steindachneri* (32 fish) and *Rasbora argyrotaenia* (24 fish). The other 3% of fish were *Gastromyzon*. Average fish densities were significantly lower in above-waterfall pools (0.02 ± 0.02 fish/m^2 versus 0.24 ± 0.27 fish/m^2; $F_{1,6} = 6.00$, $p = 0.04$). There were no fish in above-waterfalls pools at Baki and Threelan, but fish were present in above-waterfalls pools at Esu (*Gastromyzon*) and at Lower Apan (Cyprinidae).

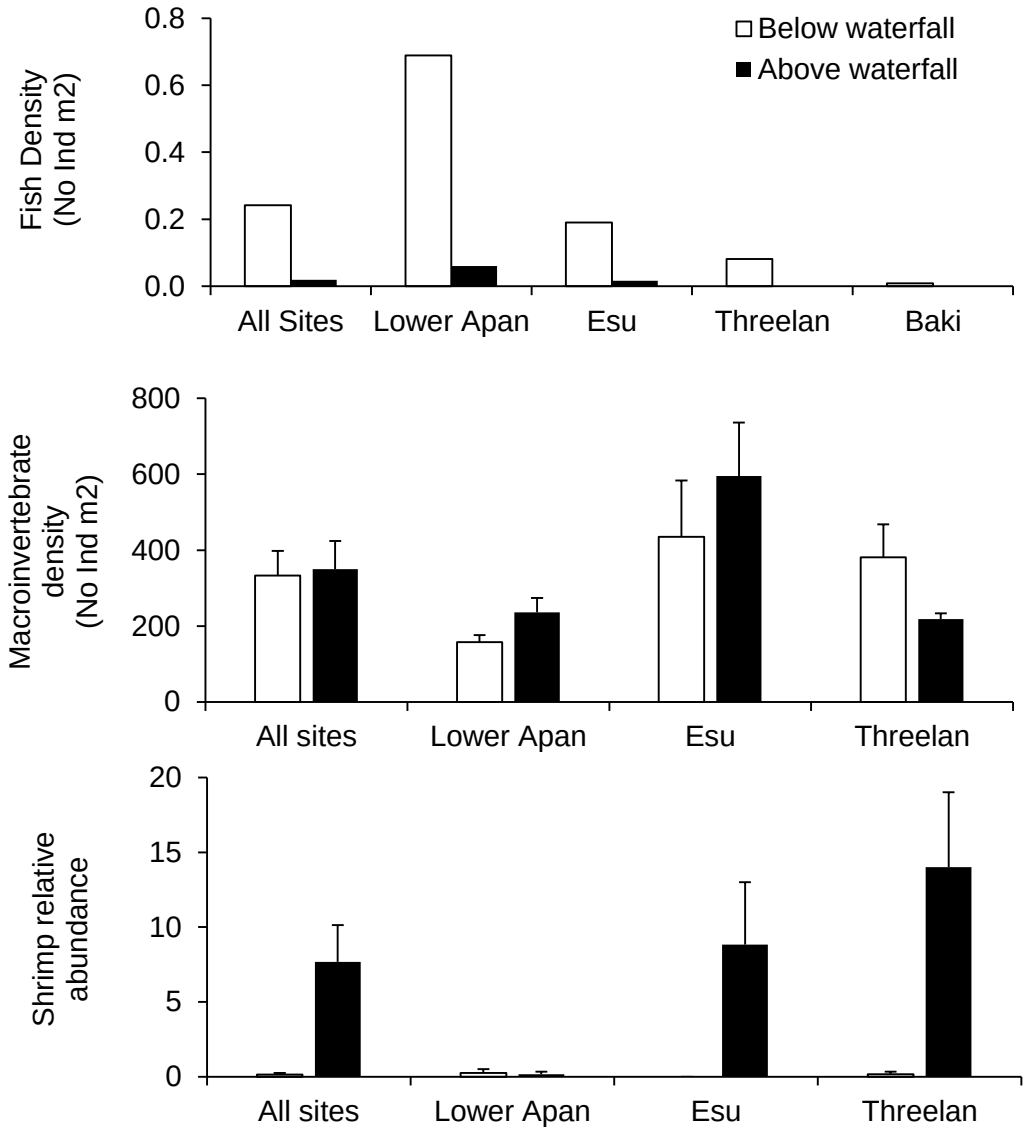


Figure 8.3. *Bar plots showing the average density of fish, shrimp and macroinvertebrates. Macroinvertebrate density was estimated using Surber samples and shrimp data from Surber and kick samples for each pool both above and below the waterfalls. Error bars represent standard errors (all below-waterfall pools, n = 8; all above-waterfall pools, n = 9; Sungai Apan Threelan, n = 6; Sungai Esu, n = 5; Sungai Lower Apan n = 6). No error bars are presented for fish density because all samples were combined in the field. There was a significant difference between above- and below-waterfall densities of fish (p = 0.04) and shrimp (p ≤ 0.01), but no difference in macroinvertebrate densities (p = 0.89). There was also a significant difference in macroinvertebrate densities among the study streams (p = 0.01)*

8.3.3 Ecosystem Function Estimates

Leaf litter decomposition: In 2013, average leaf litter breakdown rates over 7 days exhibited no variation among study streams (0.011 d^{-1} to 0.018 d^{-1}; two-way ANOVA, $F_{3, 18}$ = 2.08, p= 0.15; Table 8.2). However, over 23 days there was a significant difference among streams (0.006 d^{-1} to 0.013 d^{-1}; $F_{3, 19}$ = 6.54, p= 0.004). A Tukey's *post hoc* test shows Baki

exhibited higher breakdown rates relative to Lower Apan (p= 0.03) and Threelan (p= 0.003). In 2013, there was no difference in average leaf litter breakdown rates between above- and below-waterfall pools over 7 days (0.017 d^{-1} versus 0.012 d^{-1}, respectively; $F_{1,18}$ = 2.6, p= 0.1), but there was a difference over 23 days (0.008 d^{-1} versus 0.010 d^{-1}, respectively; $F_{1,19}$ = 9, p= 0.008) with faster decay rates occurring in below-waterfall pools (Table 8.2).

Table 8.2. *Average leaf litter breakdown rates (k_d) for pools above and below waterfalls in 2013 (over 7 and 23 days) along with macroinvertebrate (both all and non-predacious) average richness, abundance and biomass (AFDM). Leaf litter breakdown rates significantly differed among streams in 2013 over 23-day exposures ($p \leq 0.01$). Higher leaf litter decay rates were also found in 2013 over 23-day exposures below the waterfalls ($p \leq 0.01$). There was no significant difference in macroinvertebrate biodiversity or shredder diversity between pools above and below the waterfalls.*

Stream	**Exposure** (days)	**Above versus below waterfall**	**Breakdown rates** (k_d)	**Richness** (taxa/bag) All / non-pred.	**Abundance** (ind/bag) All / non-pred.	**Biomass** (mg/bag) All / non-pred.
Lower Apan	7	Above	-0.013	1.3 / 1.0	2.0 / 1.7	0.35 / 0.04
		Below	-0.010	1.7 / 1.7	1.7 / 1.7	0.45 / 0.45
	23	Above	-0.008	1.0 / 1.0	2.3 / 2.3	0.15 / 0.15
		Below	-0.008	0.0 /0.0	0.0 / 0.0	0.0 / 0.0
Baki	7	Above	-0.011	1.0 / 0.67	1.3 / 1.0	7.09 / 1.73
		Below	-0.019	3.0 / 2.33	4.3 / 3.7	0.27 / 0.15
	23	Above	-0.009	1.0 / 1.0	2.0 / 2.0	0.11 / 0.11
		Below	-0.016	2.0 / 1.7	2 .0/ 1.7	0.42 / 0.37
Esu	7	Above	-0.022	0.3 / 0.3	0.3 / 0.3	0.19 / 0.19
		Below	-0.009	3.0 / 3 .0	5.3 / 5.3	1.09 / 1.09
	23	Above	-0.008	2.3 / 2.3	4.0 / 4.0	1.56 / 1.56
		Below	-0.011	3.7 / 3.3	4.7 / 4.3	0.96 / 0.94
Threelan	7	Above	-0.013	1.5 / 1.0	2.0 / 1.5	0.23 / 0.19
		Below	-0.012	1.7 / 1.3	3.7 / 3.3	1.50 / 0.04
	23	Above	-0.005	3.7 / 3.7	10.3 / 10.3	0.26 / 0.26
		Below	-0.002	5 .0/ 4.7	5.0 / 4.7	0.88 / 0.81

In 2014, there was a difference in average leaf litter breakdown rates among the different study streams over 26 days (0.003 d^{-1} to 0.005 d^{-1}; $F_{3,26}$ = 10.35, p= 0.0001), with a Tukey's *post hoc* test showing that Esu had faster breakdown rates than Lower Apan (p= ≤ 0.01) and Threelan (p= ≤ 0.01; Table 8.3). Over 71 days, there was a difference in leaf litter breakdown among the different study streams (0.005 d^{-1} to 0.007 d^{-1}; $F_{3,25}$ = 5.43, p= 0.005; Table 8.3), with a Tukey's *post hoc* test showing that Lower Apan had slower breakdown rates relative to Esu (p= 0.04) and Baki (p= 0.007). In 2014, there was a significantly higher leaf litter breakdown rate in above-waterfall pools over 26 days (0.005 d^{-1} versus 0.004 d^{-1}; $F_{1, 26}$

= 24.80, p= ≤ 0.001) but no difference over 71 days (0.008 d^{-1} versus 0.005 d^{-1}; $F_{1, 25}$ = 3.40, p= 0.07; Fig 8.4).

Table 8.3. *Average leaf litter breakdown rates (k_d) for sites above and below waterfalls in 2014 (over 26 and 71 days) along with macroinvertebrate (both all and non-predacious) average richness, abundance and biomass (AFDM). Leaf litter breakdown rates differed among streams in 2014 over 26-day exposures ($p \leq 0.01$) and 71-day exposures ($p \leq 0.01$). There was also a significant difference in leaf litter breakdown rates between pools above and below waterfalls over 71-day exposures ($p \leq 0.01$). Over 71-day exposures, a higher abundance of macroinvertebrates accumulated in below-waterfall pools ($p = 0.002$). Higher non-predacious abundance occurred in pools below waterfalls ($p = 0.01$), whilst non-predacious invertebrate biomass was higher in above-waterfall pools ($p = 0.001$).*

Stream	**Exposure** (days)	**Above versus below waterfall**	**Breakdown rates** (k_d)	**Richness** (taxa/bag) All / non-pred.	**Abundance** (individuals/ bag) All / non-pred.	**Biomass** (mg/bag) All | non-pred.
Lower Apan	26	Above	-0.004	2.5 / 1.5	2.5 / 1.5	0.32 / 0.29
		Below	-0.006	1.0 / 0.0	3.3 / 0.0	0.76 / 0.00
	71	Above	-0.011	5.5 / 4.3	11.5 / 7.5	0.65 / 0.37
		Below	-0.001	3.3 / 2.3	5.5 / 2.0	1.10 / 0.13
Baki	26	Above	-0.016	3.8 / 2.5	9.7 / 5.8	2.16 / 2.08
		Below	-0.004	5.8 / 4.5	16.0 / 6.8	1.72 / 1.02
	71	Above	-0.002	3.5 / 2	11.0 / 2.0	0.85 / 0.40
		Below	-0.001	3.8 / 2.8	13.8 / 5.8	1.34 / 1.06
Esu	26	Above	-0.024	9.3 / 7.5	26.0 / 13.0	4.03 / 2.99
		Below	-0.005	0.0 / 0.0	0.0 / 0.0	0.00 / 0.00
	71	Above	-0.002	3.5 / 2.0	5.3 / 2.8	3.80 / 3.20
		Below	-0.006	6.0 / 5.0	29.0 / 10.2	3.59 / 2.59
Threelan	26	Above	-0.005	1.5 / 0.75	4.5 / 1.0	0.97 / 0.88
		Below	-0.004	6.0 / 4.5	13.3 / 7.5	20.67 / 17.72
	71	Above	-0.005	2.0 / 1.0	7.0 / 1.0	1.84 / 1.84
		Below	-0.005	4.0 / 2.8	17.3 / 6.8	0.83 / 0.31

Macroinvertebrate richness in the 2013 leaf litter bags ranged from 0 to 5 taxa bag^{-1} (Table 8.2) with non-predacious Chironomidae being the most frequently recorded taxa. Over the 7-day exposure there was no difference among streams in macroinvertebrate richness, abundance or biomass. However, over the 23-day exposure, there was a significant difference among the study streams in richness ($F_{3, 19}$ = 7.80, p= 0.001), but no difference in abundance or biomass. Comparisons between above- and below-waterfall pools for all streams revealed no statistical differences in richness, density or biomass (Table 8.2). Despite variation among streams, no relationships were observed between leaf litter breakdown rate and macroinvertebrate richness (R^2 = 0.02), abundance (R^2 = 0.02) or biomass (R^2 < 0.01).

Macroinvertebrate richness in the 2014 leaf litter bags ranged from 0 to approximately 9 taxa bag^{-1}, with non-predacious Chironomidae being the most frequently recorded taxa (Table 8.3). Over the 28-day exposure, there was no difference in overall macroinvertebrate richness, density or biomass among study streams or between above- and below-waterfall pools. Over the 71-day exposure, there was no difference in richness or density among study sites, but biomass did differ significantly (two-way ANOVA, $F_{3, 25}$ = 9.55, p= 0.0003), with the lowest biomass occurring at Lower Apan and then Baki. Over the 71-day exposure there was higher abundance of macroinvertebrates accumulated in the leaf litter bags of below-waterfall pools ($F_{1, 25}$ = 12.78, p= 0.002). No relationships were found between leaf litter breakdown rate and macroinvertebrate richness (R^2 = 0.17), abundance (R^2 = 0.13) or biomass (R^2 < 0.01).

Periphyton Growth: In 2013, ambient periphyton biomass averaged 2.8 ± 0.35 g m^{-2} in above-waterfalls pools and 4.3 ± 0.19 g m^{-2} in below-waterfall pools. In 2014, ambient periphyton averaged 3.4 ± 0.57 g m^{-2} in above-waterfall pools and 4.8 ± 0.37 g m^{-2} in below-waterfall pools. There were statistically higher levels of ambient periphyton in below-waterfall pools in both 2013 (one-way ANOVA, $F_{1, 26}$ = 21.90, p= ≤ 0.01) and in 2014 ($F_{1, 17}$ = 4.50, p= 0.05). However, there was no difference in ambient periphyton level among study streams.

In 2013, there was no difference in average periphyton growth rates among the study streams (0.14 ± 0.03 g m^{-2} d^{-1} to 0.40 ± 0.21 g m^{-2} d^{-1}; two-way ANOVA, $F_{3,12}$ = 1.22, p= 0.36) and no significant difference in periphyton growth rate between the above- and below-waterfall pools ($F_{1,12}$ = 1.23, p= 0.30; Table 8.4). Whilst in 2014, there was a significant difference in periphyton growth rates among the study streams (0.06 ± 0.01 g m^{-2} d^{-1} to 0.18 ± 0.03 g m^{-2} d^{-1}; $F_{3,30}$ = 6.90, p= 0.001), with Esu having the highest periphyton growth rate (Tukey's *post hoc* test; Threelan, p= 0.05; Baki, p= 0.03). Across all streams, periphyton growth rates were higher in below-waterfalls pools (two-way ANOVA, $F_{1, 30}$ = 25.70, p= ≤ 0.01).

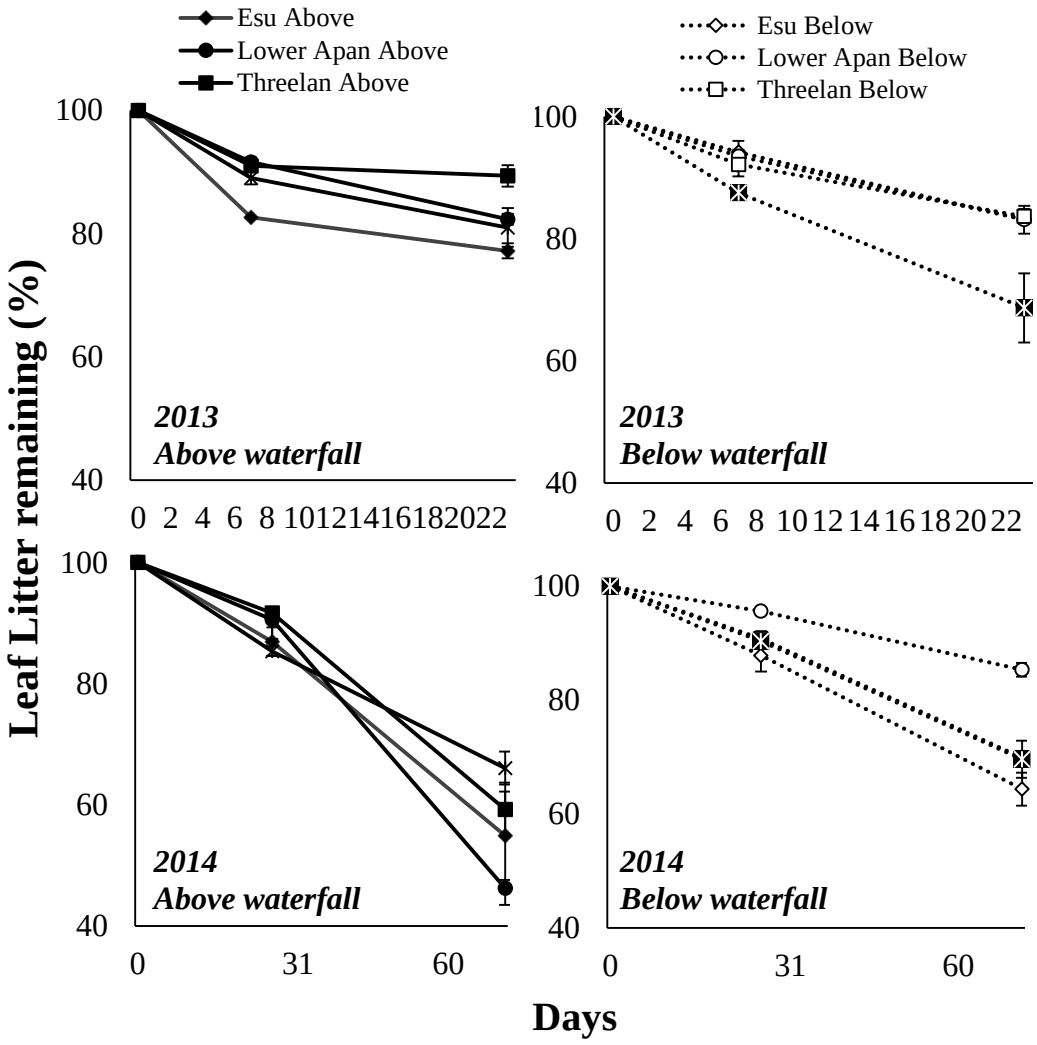


Figure 8.4. *Leaf litter decomposition in pools above and below waterfalls (top) over 23 days in 2013 and (bottom) over 72 days in 2014. Differences were significant in leaf litter breakdown rates (k_d) among streams in 2013 over 23-day exposures ($p \leq 0.01$) and in 2014 over 26-day exposures ($p \leq 0.01$) and 71-day exposures ($p \leq 0.01$). In 2013, faster decay rates occurred over 23-day exposures below the waterfalls ($p \leq 0.01$), whilst in 2014, over 26-day exposures, decay rates were higher in pools above the waterfall ($p \leq 0.01$).*

Table 8.4. *Periphyton growth rates (per day) on the rocks in pools above and below waterfalls at the four streams in 2013 (23-day exposures) and 2014 (72-day exposures). Growth rates were higher below the waterfalls in 2014 ($p \leq 0.01$) but not in 2013 ($p = 0.3$). In 2014, there was also a difference in growth rates among streams ($p = 0.02$).*

Stream, location	**Periphyton growth rate ($g\ m^{-2}\ d^{-1}$)**	
	2013	**2014**
Lower Apan, above	0.12 ± 0.01	0.05 ± 0.01
Lower Apan, below	0.23 ± 0.02	0.05 ± 0.02
Baki, above	0.10 ± 0.02	0.06 ± 0.003
Baki, below	0.12 ± 0.01	0.12 ± 0.01
Esu, above	0.22 ± 0.01	0.06 ± 0.001
Esu, below	0.17 ± 0.04	0.21 ± 0.01
Threelan, above	0.19 ± 0.01	0.04 ± N/A
Threelan, below	0.26 ± 0.01	0.13 ± 0.02

8.4 Discussion

Waterfalls can affect the distribution of aquatic animals by partitioning streams into discrete zones (Covich *et al.,* 2009; Hein & Crowl, 2010). Tropical headwater streams in northern Borneo contain a large number of shrimp and fish, with waterfalls acting as a semi-permeable filters that create different aquatic communities in reaches above and below waterfalls (Covich *et al.,* 2009; Hein & Crowl, 2010). In my study streams, I found below-waterfall pools supported a higher density of fish and significantly higher ambient periphyton levels in 2013 and 2014 (Table 8.5). Higher periphyton growth levels were also found in below-waterfall pools in 2014 (Table 8.5). Above-waterfalls pools support both a higher abundance and biomass of shrimp but there was no difference in macroinvertebrates between above- and below-waterfall pools, and no consistent statistical difference in leaf litter decomposition rates in 2013 or 2014 (Table 8.5). These findings illustrate how geomorphology can isolate biotopes, creating discontinuities in streams that affect community structure, specifically with respect to fish and shrimp. However, the corresponding impact on ecosystem functions is less straightforward, suggesting differing degrees of influence of biotic and abiotic factors on ecosystem functions.

Table 8.5. *Survey summary showing significant differences in animal communities and ecosystem functions among the study streams and between the above- and below-waterfall pools.*

Parameter	All streams *P*-value	Above & below waterfall *p*-value	Notes
Fish density	0.48	0.04	*Gastromyzon* present above waterfalls at Esu (1) and Cyprinidae at Lower Apan (3)
Shrimp density	0.18	<0.01	Only *Macrobrachium* present
Macroinvertebrate density	0.01	0.89	Lower Apan exhibited the lowest density
Leaf litter 2013- 7 days	0.15	0.1	Faster decay rates in pools below waterfalls over 23 days
Leaf litter 2013- 23 days	<0.01	<0.01	
Leaf litter 2014- 26 days	<0.01	<0.01	Faster decay rates in pools above waterfalls over 26 days
Leaf litter 2014- 71 days	<0.01	0.07	
Periphyton (ambient) 2013	0.12	<0.01	More periphyton below waterfalls
Periphyton (ambient) 2014	0.50	0.05	
Periphyton growth 2013	0.36	0.30	Esu had the fastest periphyton growth and fastest growth rate below the waterfall
Periphyton growth 2014	<0.01	<0.01	

8.4.1 Biodiversity in above- and below-waterfall pools

According to Choy & Chin (1994) the fish observed in the study streams were some of the most common in the Temburong catchment. As expected, the highest densities of fish were found in below-waterfall pools. Waterfalls (>6 m) acted as a block for fish at Esu, Threelan and Baki, likely creating a refuge for shrimp in the pools above (Covich *et al.*, 2009; Hein & Crowl, 2010). Choy (1996), who studied the distribution ecology of fish in the Ulu Temburong National Park reported similar findings, with no Cyprinidae taxa recorded above any waterfall greater than 5 m. The *Gastromyzon* fish were an exception; these herbivorous fish have suckers along their bodies and are therefore able to ascend vertical bedrock (Lucas *et al.,* 2001). However, in spite of this trait, surprisingly few *Gastromyzon* were observed in pools above waterfalls. This may be the result of the sampling design, as many *Gastromyzon* species inhabit faster flowing biotopes (Sheldon, 2011), which were not sampled in my study.

Macrobrachium (shrimp), which were collected in all sampled streams (Lower Apan, Esu and Threelan), are important members of aquatic communities. However, their impact on benthic environments have mainly been studied in Neotropical streams, with only a few studies conducted in Asia (e.g. Mantel *et al.,* 2004). In the Neotropics, *Macrobrachium* have been found to exhibit aggressive behaviour, which can cause other shrimp to take shelter (Crowl & Covich, 1994; March *et al.,* 2001). This behaviour may explain the absence of other shrimp taxa sampled in our study sites. There were no significant differences in shrimp biomass and density among the streams. Among all pools above the waterfalls there were significantly higher shrimp densities (Fig 8.3). These results concur with similar findings in Puerto Rico, where shrimp occur in high abundances in above-waterfall pools (Pringle *et al.,* 1993; March *et al.,* 2002; Covich *et al.,* 2009).

However, the high abundance of shrimp in above-waterfall pools did not appear to have a significant impact on macroinvertebrate communities, with the NMDS results finding no difference in community patterns in macroinvertebrates in pools-above and- below the waterfall. In the headwater streams of Puerto Rico, Ramirez & Hernandez-Cruz (2004) conducted a shrimp exclusion experiment, which involved *Macrobrachium* and exhibited mixed impacts on aquatic insects (i.e. effects of shrimp were negligible in some streams). In another study in Puerto Rico, March *et al.,* (2001) found that the impact of shrimp exclusions on insect biomass varied depending on the genus of shrimp. In the high altitude pools, exclusion of shrimp, which included *Atya* spp. and *Xiphocaris elongata*, resulted in significantly lower insect biomass. In contrast, exclusion of *Macrobrachium* spp. in the low altitude pools promoted higher proportions of insect biomass. In my study, the low numbers of *Macrobrachium* in below-waterfall pools had no apparent effect on macroinvertebrate biomass, while in Costa Rica, fish and shrimp significantly reduced aquatic insects with combined effects greater than the sum effect of both groups individually (Pringle & Hamazaki, 1998). These studies conducted in the Neotropics suggest that freshwater shrimp have an important role in structuring benthic community composition, but their effects vary and may depend on the presence or absence of other biota (March *et al.,* 2002). In Hong Kong, Mantel *et al.,* (2004) found no effect of the removal of *Macrobrachium* on aquatic insects; however, the authors were unable to determine the reason there was no effect. One explanation is that the macroinvertebrates consistently evaded the fish and shrimp predators; this has been shown in studies that artificially doubled the natural densities of fish and failed to observe a decrease in some invertebrate taxa (Gilliam *et al.,* 1989; Dudgeon, 1991). My study also lacks a consistent pattern in macroinvertebrate diversity between above- and below-waterfall pools,

suggesting that communities of shrimp and fish do not influence these biotic patterns; however, it is possible that the presence of fish and shrimp predators is substitutable and thus functionally redundant (*sensu* Ho & Dudgeon, 2016).

In Northern Australia, Garcia *et al.,* (2015) found that top-down effects on macroinvertebrates were context dependent with respect to factors such as benthic habitat, stream velocity and community structure, each of which influenced invertebrate diversity. Ulu Temburong has frequent flood events; for example, after a storm it is not uncommon for the level of Belalong to rise by 1 m in a 30-minute period (Cranbrook 1993). These 'flashy flows' may be more important in structuring macroinvertebrates than the presence of shrimp or fish, scouring out habitats and physically removing taxa (Bond & Downes, 2000). The hypothesis that predation and competition are less important in highly disturbed streams and rivers has been suggested for systems in other parts of the world (Bishop, 1973; Peckarsky *et al.,* 1990; Yang & Dudgeon, 2010). Flash floods can create relatively unpredictable systems (Boulton *et al.,* 2008), creating a continuum of conditions, which may result in two distinct environments. For example, during low flows, tropical streams are complex systems exhibiting a mix of flow biotopes (i.e. pools, riffles and cascades) and functional habitats (i.e. wood debris, leaf litter, cobbles and gravel; *sensu* Harper *et al.,* 1995, Harvey *et al.,* 2008); however, during a flood event, these streams become homogeneous as water rises to form a uniform flood biotope.

8.4.2 Ecosystem Function

It was assumed that differences in fish and shrimp densities would affect macroinvertebrate patterns along with leaf litter decomposition rates, ambient periphyton levels and growth rates. Specifically, past studies in the tropics have suggested that leaf litter decomposition can be increased by higher shrimp densities (March *et al.,* 2001; Wright & Covich, 2005), and herbivorous fish can increase periphyton levels (Pringle & Hamazaki, 1997). I found ambient periphyton and growth levels associated with higher fish densities, while leaf litter decomposition rates were unaffected by varying fish or shrimp densities.

The observed leaf litter decomposition rates (Tables 8.2 and 8.3) were in the range of other tropical studies reported by Wantzen *et al* (2008) with processing rates extending from 0.001 d^{-1} (Mathooko, 1998; Rueda-Delgado *et al.,* 2006) to 0.789 d^{-1} (Mathooko & Kariuki, 2000; Tables 8.2 and 8.3). However, the lack of a correlation between fish, shrimp, and macroinvertebrates parameters and break down rates suggests that leaf litter loss in my study sites was caused by other factors. Microbial processing has been suggested to be more

important in the tropics compared to temperate regions (Irons *et al.,* 1994). However, the importance of microbes to the decomposition of leaf litter is poorly understood, especially in the tropics (Wright and Covich, 2005), and thus, more research is required to understand the role of bacteria and fungal on breakdown rates. Mechanical abrasion from frequent floods in the catchment can affect leaf litter break down, which has been demonstrated in other tropical studies (Pearson *et al.,* 1989). Fast flows, which occur frequently in streams within Ulu Temburong, have been reported to cause physical abrasion of leaf litter, breaking them down into smaller parts and therefore influencing breakdown rates (Wantzen *et al.,* 2008).

In this study, there were no significant differences in leaf litter decay between above- and below-waterfall pools. Many streams that flow through dipterocarp forests, such as my study sites, have very high tree diversity and leaves that are high in lignin and low in protein (Yule *et al.,* 2009); such leaves possess an unappealing combination of traits for shredders. Landeiro *et al* (2008) excluded fish and shrimp (mainly *Macrobrachium*) from leaf litter bags and found greater leaf litter breakdown in the control relative to the exclusion area. This could be linked with higher insect shredder abundances; however, there was no difference in shredder abundance between treatments, and the results were inconclusive. In spite of more recent research suggesting insect shredders do exist in tropical streams (Cheshire *et al.,* 2005; Masese *et al.,* 2014), my regression analysis showed no correlation between leaf litter breakdown rates with macroinvertebrate diversity (in neither 2013 nor 2014). In my study, some leaf litter bags lacked any macroinvertebrates, for example, those in Lower Apan (e.g. the 2013, 23-day exposure bags in the below-waterfall pools; Table 8.2), suggesting that leaf litter is an unappealing food source for many tropical macroinvertebrates.

Periphyton growth rates appear to be low compared to ambient periphyton levels, which is unsurprising because of the abundant herbivorous grazers and frequent scouring flows. However, some tropical fish have been found to be vitally important in increasing periphyton growth rates (Pringle & Hamazaki, 1997; Moulton *et al.,* 2010), which may explain high levels of ambient periphyton in below-waterfall pools and low periphyton growth rates at the above-waterfall pool of Apan Threelan (0.04 g m^{-2} d^{-1}), where no fish were present. Overall higher levels of ambient periphyton were correlated with the distribution of fish (Table 8.5). Pringle & Hamazaki (1997) made similar findings; they observed fish influencing algal community composition, whereas shrimp had no significant effect. Studies have found herbivorous fish to be efficient in cleaning rock surfaces of deposited sediment after storm

events, therefore helping to increase periphyton levels (Pringle & Hamazaki, 1997). This is important in tropical streams, such as my study streams, where fast flows are frequent and can both cause physical scouring and layer rocks in fine sediment (Wantzen *et al.,* 2008). However, rates of accrual including grazing and scouring losses of periphyton among our study streams are not known.

Although many of the fish sampled in my study streams were not herbivorous, the literature contains studies in which even predacious fish have been shown to create trophic cascades in tropical streams. Moulton *et al* (2010) found that predacious fish inhibit shrimp and baetid mayflies from grazing in two Neotropical streams, resulting in higher levels and qualities of periphyton in pools containing fish. However, other experiments have found that top-down effects of fish on algae have less impact relative to bottom-up factors such as increases in nutrient levels (Garcia *et al.,* 2015; Ho & Dudgeon, 2016). Interestingly, Ho & Dudgeon (2016) found no impact of high numbers of fish and shrimp on algal biomass or periphyton accumulation in three Hong Kong streams. This was suggested to be caused by the removal of macroconsumers, which may have reduced nutrient levels and therefore contributed to the apparently weak top-down effect on fish and shrimp (Ho & Dudgeon, 2016). Other factors, such as variation in sunlight across sites, may also effect periphyton levels; this requires further investigation in my study sites. However, as my experiments were conducted in pools directly above-and-below the waterfall, they experienced similar light levels.

8.5 Conclusion

As hypothesised, this study clearly illustrates that waterfalls affect habitat patterns of fish and shrimp, with waterfalls (generally >5 m in height) acting as a barrier for fish. This natural habitat fragmentation does impact some ecosystem functions, specifically primary productivity. Further studies must be conducted to understand the effects of other factors, such as frequent scouring flows, which can have greater impacts than biotic factors. These findings suggest that streams divided into naturally discrete units by waterfalls have considerable ecological and conservation significance. Recent land-use changes caused by the widespread growth of the palm oil industry in Southeast Asia have increased the urgency of identifying and studying the remaining pristine rivers and streams. Understanding how natural discontinuities, such as waterfalls, can affect habitat patterns and ecosystem functions is vitally important to the success of management and conservation of these systems.

Chapter 9: Macroinvertebrate trophic structure on Waterfalls in Borneo

9.1 Introduction

Waterfalls have received limited attention with only a few published papers to date (Rackemann *et al.*, 2013; Clayton and Pearson, 2016). Research conducted on waterfalls in the tropics have generally focused on them acting as a longitudinal block for fish movement, creating an upstream refugia for taxa including shrimp and tadpoles (Covich *et al.,* 2009*;* Hein and Crowl, 2010; Torrente-Vilara *et al.,* 2011*;* Kano *et al.,* 2012), with very little information on the waterfalls themselves (however see Clayton and Pearson, 2016; Rackemann *et al.,* 2013; Clayton and Pearson, 2016). This lack of research may be explained by several factors including waterfalls being perceived to be biological dead zones, too complex to sample effectively and being inherently dangerous environments due to fast flows and sheer drops (Rackemann *et al.*, 2013). Waterfalls are defined by their bedrock substrate and fast flows (Newson and Newson, 2000) and are unique compared to other biotopes because of their sheer shallow flows and lack of connectivity to the hyporheic zones (Clayton, 1995). In addition, these biotopes are formed and maintained by erosive processes that occur over geologic periods creating stable conditions, which are relatively uniform across a range of discharges (Clayton, 1995). In comparison, other biotopes such as pools and riffles change daily, frequently re-shaped by erosion and deposition events, whereas waterfalls are more stable (Clayton and Pearson, 2016).

Although waterfalls have stable substrate, resident taxa have to be specialized to live in an environment with such turbulent and relatively fast velocities and no hyporheic zone (Clayton and Pearson, 2016; Hart and Finelli, 1999). Hora (1930) and Nielsen (1950) were the first authors to officially describe rheophilic taxa, they noted some of the taxa's adaptations to withstand fast current velocity including hooks, suckers and modified legs (Fig 9.1). Taxa have to be physically adapted to enable them to move around the rock without being washed away or to fit into cracks and crevices (Clayton, 1995). For example Furtado's (1969) analysis of odonates in a Malaysian stream found that some dragonflies managed to inhabit trailing plants and accumulated debris irrespective of water velocity, assisted by its body's morphology covered in spines aiding attachment.

Taxa adapted to waterfalls can benefit because these biotopes are relatively free from fish and shrimp, which have been found to be important in determining insect assemblage structure (March *et al.,* 2002*;* March and Pringle, 2003; Ramirez and Hernandez-Cruz, 2004). Fish and shrimp impact insects via predation and competition for food resources, breaking up and consuming leaf litter and grazing periphyton (Pringle and Hamazaki, 1997; Crowl *et al.,* 2001; Flecker and Taylor, 2004; Ramirez and Hernandez-Cruz, 2004; Coughlan *et al.,* 2010). In addition, fish and shrimp can occur in high densities creating a competition for space with invertebrates. Waterfalls are therefore unique biotopes for invertebrates as they can reside on the substrate without top down disturbance. It is predicted that the difference in community structure on waterfalls, with the absence of fish and shrimp, will create a distinctive food web compared to other tropical biotopes.

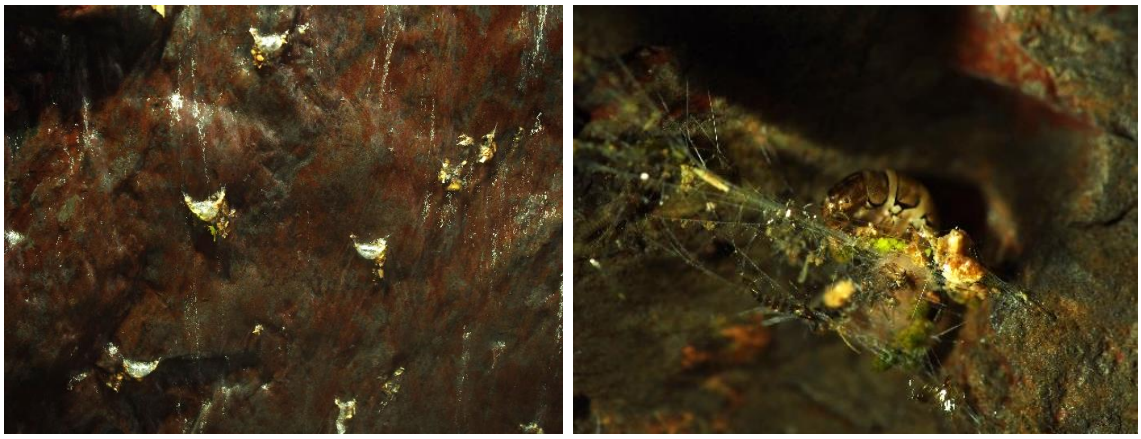


Figure 9.1. *Hydropsychidae nets on a waterfall (left photo) and a close up image of a Hydropsychidae in its net (right photo)*

Food web studies using stable isotope analysis in tropical streams have focused on pool and riffle biotopes dominated by shrimp and fish (for example see: Brito *et al.*, 2006; Li and Dudgeon, 2008; Coat *et al.*, 2009). Some of these studies have found invertebrates in the tropics to depend more on algal based food compared to leaf litter (Brito *et al.,* 2006; Lau *et al.,* 2009a). Even in shaded reaches, tropical food webs appear to be mainly algae based (Bunn and Arthington, 2003; March and Pringle, 2003; Brito *et al.*, 2006). Gaps in the canopy often occur near to waterfalls as a result of the high rates of erosion below the waterfalls, which create wide plunge pools (Odland *et al.*, 1991). This canopy gap enables more light to reach the waterfall substrate promoting periphyton growth. In contrast, standing stocks of benthic organic matter tend to be low on waterfalls with export due to fast flows, channel gradient

and the lack of retentive structure. This reduces the detrital food sources for macroinvertebrates. Although waterfalls are discrete units they are not separated from the river continuum with resident filter feeders, such as Simuliidae and Hydropsychidae, dependent on seston and organic matter from upstream. However, the strength of upstream-downstream linkages have been debated with Huryn *et al.,* (2002) suggesting that local factors are still more of a determinant of trophic resources.

Investigations into food webs in tropical streams have mainly focused on pools and riffles. My study sought to examine macroinvertebrate trophic structure on waterfalls using both gut contents analysis (GCA) and stable isotope analysis (SIA). It is hypothesised that due to the steep gradient of waterfalls and low retention of terrestrial based resources, the abundant basal food resources of macroinvertebrates would be periphyton.

9.2 Methods

9.2.1 Study Sites

Ulu Temburong National Park in Brunei Darussalam (550 km^2) is an expansive pristine tropical rainforest with free flowing rivers making it an excellent location for freshwater research (Sheldon, 2011). The landscape in the national park changes over a relatively short distance (<35 km) from steep mountainous reaches (1,850 m a.s.l. at Bukit Pagon and 913 m a.s.l. at Bukit Belalong) to placid lowlands (KBFSC 30 m a.s.l.) (Dykes, 1994). This creates a geomorphic template for many waterfalls and cascades, therefore a suitable region for this study (Fig 9.2).

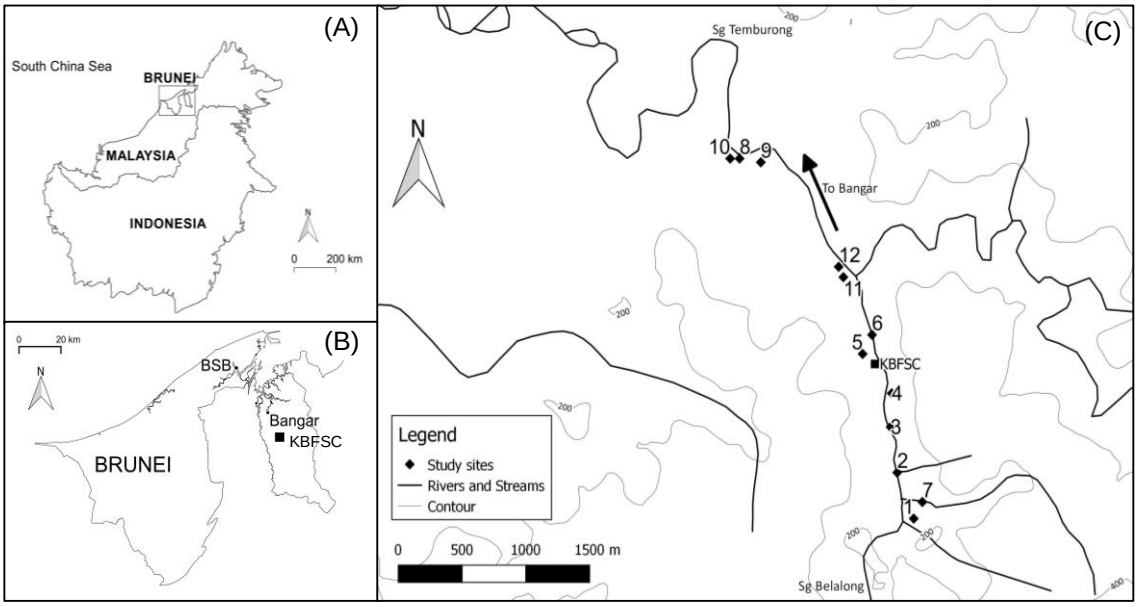


Figure 9.2 *Map showing the location of Brunei Darussalam in northern Borneo (A). Brunei Darussalam is split into two parts with this study being conducted in the Temburong district, the eastern section of Brunei (B). The twelve waterfalls are situated on tributaries off of the two main rivers Sungai Belalong and Sungai Temburong (C).*

Brunei is in the tropics and weakly influenced by the South East Asian monsoon however weather in Ulu Temburong is highly variable in nature with localised storms (Dykes, 1996). Sampling took place in June and July (2014) when rainfall and stream discharges are generally low making access to the waterfalls safe (Edwards, 1994). Individual waterfalls on twelve tributaries to Sungai Temburong or Sungai Belalong were investigated (Fig 9.2). Waterfalls selected to be sampled were the first upstream after the confluence with the main rivers (Fig 9.2).

9.2.2 Geomorphic Measurements and Functional Habitats

Waterfall measurements included the width and length being measured with measuring tape, whilst slope angle was measured with a clinometer. In the cases where waterfalls were very steep the length was estimated. The presence and distribution of functional habitats (e.g. moss, leaf litter, wood debris) were recorded (for an explanation of functional habitats see Harper *et al.,* 1995). Descriptions of substrate heterogeneity were noted along with photos of the waterfalls to enable qualitative image analysis of waterfall substrate complexity.

9.2.3 Macroinvertebrate trophic structure

The common and frequently occurring taxa on the waterfalls including Heptageniidae, Blephariceridae, Simuliidae, Hydropsychidae, and Buccinidae were sampled by hand selection. Trophic structure was then evaluated using gut contents analysis (GCA) and stable isotope analysis (SIA).

Gut Contents Analysis: Gut contents analysis (GCA) has been used frequently to investigate benthic macroinvertebrate resource utilization (Rosi-Marshall and Wallace, 2002; Li and Dudgeon, 2008). Animal tissue was present in the guts of some macroinvertebrates, this information was useful to support the SIA results with the added benefit of being able to sometimes identify the prey. Three individuals of each of the taxa were collected for GCA. Macroinvertebrates were preserved in 70% ethanol and shipped back to the UK. In the laboratory foreguts were removed from the taxa under a dissecting microscope, the contents were taken out and mounted in Euparal on a microscope slide (Layer *et al.*, 2012). The contents were identified at 400-1,000x magnification into five categories; coarse particulate organic matter (CPOM) including leaf litter and wood, algae (diatoms, green alga), fungal hyphae and conidia, animal tissue and amorphous detritus classified due to the lack of any defined cellular structure; from Yule *et al.,*(2010). Percentage of gut contents analysis was then calculated.

Stable Isotope Analysis: Due to the small size of tropical macroinvertebrates (Jacobsen *et al.*, 2008) gut contents can be difficult to identify therefore SIA was additionally used to investigate the macroinvertebrate basal food resources. Stable isotopes have been widely used to evaluate energy flow and trophic structure in a range of food webs (Peterson and Fry, 1987). Stable carbon and nitrogen isotope ratios are a useful tool to calculate what has been assimilated by the macroinvertebrates and to determine the relative importance of basal food resources in the food web. Carbon isotope ratios have been found to be effective in distinguishing between autochthonous (aquatic) and allochthonous (terrestrial) food sources, with the former having higher $\delta^{13}C$ values (Fry, 1991; Hershey and Peterson, 1996). Nitrogen isotopes have been used to provide information about trophic levels with $\delta^{15}N$ increasing with each trophic level.

Taxa were collected for stable isotope analyses using the same methods as gut contents analysis (see above). From 3-50 individuals per taxa were selected to ensure enough sample material for analysis. All taxa were left in stream water for 12 hours to promote gut

clearance. Putative food sources (leaf litter and periphyton rock scrubs) were also taken at each waterfall. All samples were dried at 70 degrees Celsius for a 24hour period before being transported back to the UK. Samples were weighed to approximately 0.5mg and loaded into 5x7mm tin capsules. Leaf litter was ground into a fine powder. Three replicates were used for macroinvertebrates and leaf litter, whilst five replicates of periphyton (due to the increased variability).

Measurements were carried out at the NERC Life Sciences Mass Spectrometry Facility (LSMSF) in East Kilbride, using an Elementar Pyrocube elemental analyser coupled with a Thermo Fisher Scientific Delta Plus XP mass spectrometer. Laboratory standards Fluka gelatin, Sigma alanine and Sigma glycine (Sigma-Aldrich Company Ltd, Gillingham, UK) were repeated with every 10 samples and were used to correct for linearity and instrument drift over a 22 hour analytical run. The isotope ratios for the lab standards are determined relative to a range of International standards from IAEA (Vienna, Austria) and USGS (Reston, VA, USA). Isotope ratios are expressed in the δ (delta) notation in parts per million (‰): $\delta X = [(R_{sample}/R_{standard})-1]$ where X = ^{15}N or ^{13}C and R = the ratio of $^{15}N/^{14}N$ or $^{13}C/^{12}C$ isotopes in a given sample relative to AIR for nitrogen and PDB for carbon. The analytical precision for carbon and nitrogen isotope measurements was better than 0.3‰.

Data Analysis: Analysis of variance (ANOVA) was used to assess differences in stable isotope compositions for both food sources and consumers. It was also used to assess differences among all the waterfalls. Tukey Post Hoc test was used after the ANOVA to examine specific differences (Thomas *et al.*, 2013). The statistical computing environment R (R Core Team 2013) was used to conduct the ANOVAs. The focus of the study was to examine allochthonous (leaf litter) and autochthonous (periphyton) food sources on the waterfalls. As not all food sources were sampled (e.g. moss, seston, fungi), a mixing model was not applied to the data.

9.3 Results

9.3.1 Geomorphic Measurements and Functional Habitats

The twelve waterfalls had a range of average slope angles, varying from 16° (waterfall 11) to a maximum average of 53° (waterfall 1). Lengths of the waterfalls varied from 4.5m (waterfall 11) to a maximum of 20.4m (waterfall 8-Table 9.1). All waterfalls were covered in periphyton with at least a few leaves present. Half of the waterfalls had wood debris, which

varied in size from twigs to large tree trunks. Landslides and tree falls are not uncommon in Ulu Temburong National Park (Dykes, 1994), with much of the debris ending up in the streams and rivers. Frequently, large tree trunks become lodged in between the bedrock and the narrow bankful widths, this occurred at waterfalls 1, 4 and 7. Based on observations in the field and subsequent qualitative image analysis, waterfalls varied in substrate complexity. Some of the waterfalls consisted of smooth rock while others were more complex with a mix of ridges (millimetres to centimetres), ledges and undercutting.

Table 9.1. *Physical measurements of waterfalls including average slope angle and length. Presence and absence (1Vs 0) of functional habitats include leaf litter, wood debris and moss. ~ indicates estimated values.*

Waterfall	Slope angle (degrees)	Length (m)	Leaf litter	Wood Debris	Moss
1	53	5.7	1	1	0
2	26	4.93	1	0	0
3	20	6.16	1	0	0
4	50	15	1	1	1
5	47	7.2	1	0	1
6	39	7	1	0	1
7	47	~6	1	1	1
8	35	20.4	1	0	0
9	27	~13	1	0	0
10	16	9.13	1	0	0
11	40	4.47	1	0	1
12	48	16.2	1	1	0

9.3.2 Macroinvertebrate trophic structure

The sampled macroinvertebrates included Simuliidae (filtering collector), Hydropsychidae (omnivorous filtering collector), Blephariceridae (herbivorous grazer), Heptageniidae (herbivorous grazer) and Buccinidae (predator). At waterfalls 8 and 11 there were no Blephariceridae and no Buccinidae at waterfall 8.

Gut Contents Analysis: Amorphous detritus was most abundant in macroinvertebrate guts, ranging from ~45% to ~100% (Fig 9.3). CPOM was the next abundant ranging from 0 to 30% and algae varied from 0 to ~20% (Fig 9.3). Animal tissue varied from 0 to ~7% and the least abundant matter in the macroinvertebrate guts was fungal hyphae and conidia varying from 0 to a maximum average of less than 5% (Fig 9.3). Blephariceridae contained 100% amorphous detritus. Simuliidae contained a high percentage of amorphous detritus (~95%), but in addition included CPOM, algae and in one individual an insect head was

observed (Waterfall-9; Fig 9.3). Heptageniidae contained a high percentage of amorphous detritus, but also contained algae and fungal hyphae and conidia (Fig 9.3). Unlike the other taxa, Hydropsychidae had stomach contents that were not dominated by amorphous detritus and there was a more even mix of all observed food types (Fig 9.3).

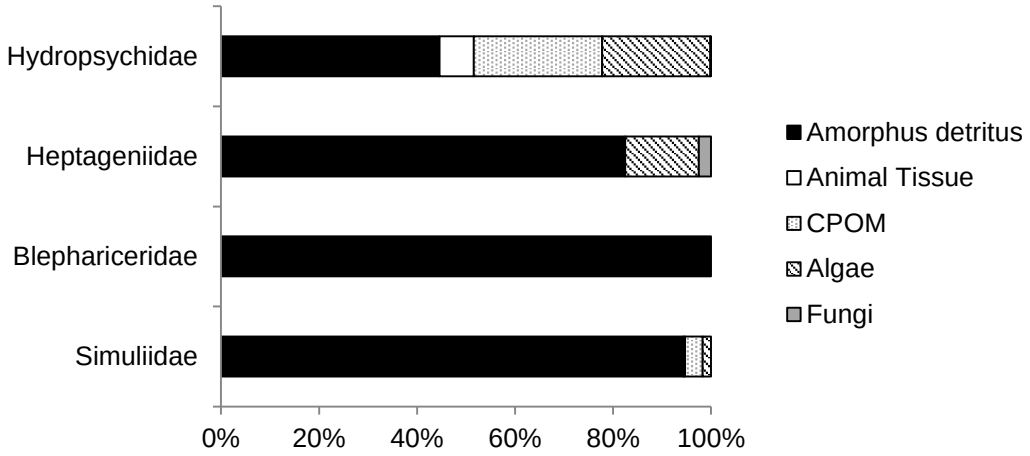


Figure. 9.3. *Average percent contributions of stomach contents including amorphous detritus, animal tissue, CPOM, algae and fungal hyphae and Conidia in the five macroinvertebrates Hydropsychidae, Heptageniidae, Blephariceridae and Simuliidae.*

Stable isotopic composition of food sources: Periphyton had less depleted ^{13}C values ($\delta^{13}C_{periphyton}$= -30.06 ± 2.54 ‰) compared to leaf litter ($\delta^{13}C_{leaf\ litter}$ = -32.97 ± 1.58 ‰), making it possible to distinguish between these basal food resources (one-way ANOVA, $F_{1, 83}$=37.7, p=<0.01; Table 9.2; Appendix Chapter 9). The $\delta^{15}N$ (‰) value for periphyton (1.29 ± 0.78 ‰) and leaf litter (-0.91 ± 0.99 ‰) was also distinct (one-way ANOVA, $F_{1, 83}$=142.6, p=<0.01), with periphyton having significantly higher $\delta^{15}N$ values than leaf litter. The C: N values were higher for the leaf litter (49.27) than for periphyton (5.37). The lower ratio indicates there is a higher proportion of nitrogen in the sample suggesting higher protein digestibility and therefore of higher nutritional value to the consumers (Giller and Malmqvist, 1998; Table 9.2).

Table 9.2. *Average carbon and nitrogen isotopic values of basal food sources and consumers (mean + SD).*

	n	**C:N**	**δ^{13}C (‰): Mean ± SD**	**δ^{15}N (‰): Mean ± SD**
Basal food sources				
Leaf litter	36	49.27	-32.97 ± 0.26	-0.91 ± 0.16
Periphyton	57	5.37	-30.06 ± 0.34	1.29 ± 0.10
Consumers				
Heptageniidae	36	4.56	-29.47 ± 0.33	1.06 ± 0.13
Blephariceridae	30	4.50	-31.45 ± 0.42	1.50 ± 0.12
Simuliidae	36	4.30	-28.14 ± 0.09	2.16 ± 0.06
Hydropsychidae	36	4.53	-28.58 ± 0.14	3.07 ± 0.08
Buccinidae	33	3.92	-26.18 ± 0.16	5.48 ± 0.12

Stable isotopic compositions of consumers: The δ^{13}C values for Heptageniidae at the 12 waterfalls varied from -35.51 ‰ to -26.83 ‰, while Belphariceridae ranged from -35.68 ‰ to -27.41 ‰ (Table 9.2; Fig 9.4; Appendix Chapter 9). Simuliidae varied from -29.29 ‰ to -27.33 ‰ and Hydropsychidae δ^{13}C values ranged from -31.12 ‰ to -27.2‰. Buccinidae ranged from -28.03 ‰ to -24.04 ‰ (Table 9.2; Fig 9.4). There was a statistical difference between the δ^{13}C values of all consumers (one-way ANOVA, $F_{4, 151}=55.5$, $p<0.01$), but the Tukey post hoc test showed no difference between Heptageniidae and Blephariceridae. Mean δ^{15}N values of Heptageniidae ranged from a minimum of -0.56 ‰ to 2.51 ‰, Blephariceridae varied from 0.51 ‰ to 2.64 ‰ and Simuliidae varied from 1.65 ‰ to 3.04 ‰ (Table 9.2). Hydropsychidae ranged from 2.26 ‰ to 4.50 ‰ and Buccinidae ranged from 4.12 ‰ to 6.69 ‰ (Table 9.2). There was a statistical difference between the consumers in δ^{15}N values (one-way ANOVA, F $F_{4, 151}=275.7$, $p=<0.01$). However, the Tukey post hoc test showed no difference in δ^{15}N values between Heptageniidae and Blephariceridae ($p=0.34$), and between Simuliidae and Hydropsychidae ($p=0.8$).

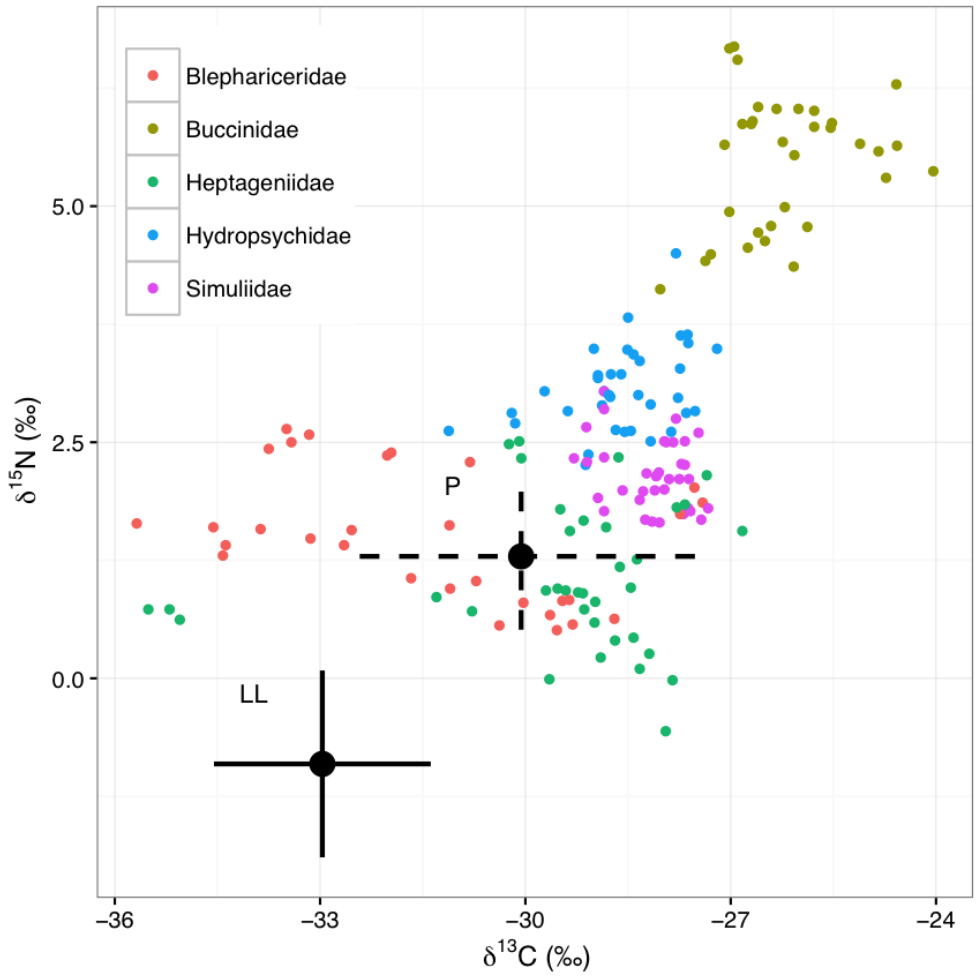


Figure 9.4. *Mean δ 13 C and δ 15 N values of basal resources leaf litter (LL) and periphyton (P) for the dominant macroinvertebrates found on the waterfalls in Ulu Temburong National Park. Macroinvertebrates include Buccinidae, Blephariceridae, Heptageniidae, Hydropsychidae and Simuliidae. Error bars indicate +1 SD.*

Potential food sources of consumers using GCA and SIA: Most Heptageniidae had $\delta^{13}C$ values that corresponded to periphyton (Table 9.2; Fig 9.4). However at waterfall 7, Heptageniidae had a $\delta^{13}C$ value of approximately -27 ‰ and at waterfall 10 the $\delta^{13}C$ values were very depleted at approximately -35 ‰ (Table 9.2; Fig 9.4). GCA found Heptageniidae consumed CPOM, algae and, fungal hyphae and conidia, the latter food source may explain the 'unknown' $\delta^{13}C$ values. At most waterfalls Blephariceridae matched periphyton and leaf litter, however at waterfall 10 $\delta^{13}C$ values were depleted with $\delta^{13}C$ values less than -35 ‰. Due to the small size of the Blephariceridae the GCA could not be used to show any further resolution, with gut material classed as amorphous detritus. Simuliidae $\delta^{13}C$ values matched periphyton. However, there were some Simuliidae at waterfall 11 that had $\delta^{13}C$ value of approximately -27 ‰, values that are on the edge of the periphyton $\delta^{13}C$ (Fig 9.4).

Observation of the GCA didn't show any further resolution (Fig 9.3). Hydropsychidae $\delta^{13}C$ values were similar to periphyton, along with another food source with $\delta^{13}C$ of approximately -27 ‰ at waterfall 8 and 11. GCA of Hydropsychidae contained animal tissue, fungal hyphae and conidia, CPOM and algae. Buccinidae $\delta^{13}C$ values did not match periphyton or leaf litter apart from at waterfall 9 where $\delta^{13}C$ values corresponded to periphyton. It was not possible to differentiate food types in the Buccinidae gut contents as it was pulverized.

The range of $\delta^{15}N$ values indicate that macroinvertebrates on the 12 waterfalls fed at different trophic levels (Fig 9.5). Heptageniidae had the most depleted ^{15}N with a value of 1.06 ± 0.77 ‰ and Blephariceridae had a $\delta^{15}N$ value of 1.50 ± 0.68 ‰ (Table 9.2; Fig 9.4). Simuliidae and Hydropsychidae $\delta^{15}N$ values were higher, Simuliidae had average $\delta^{15}N$ value of 2.16 ± 0.36 ‰ and Hydropsychidae $\delta^{15}N$ value was 3.07 ± 0.46‰ (Table 9.2; Fig 9.4). This indicates that some of their diet was composed of animal tissue. GCA showed that animal tissue was present in the stomach contents of Simuliidae, although this was only present in taxa at waterfall 9. Hydropsychidae had animal tissue in gut contents for half of the waterfalls including 2, 3, 7, 8, 9 and 12. Whilst Buccinidae was distinctly predacious, having the most enriched ^{15}N with values of 5.48 ± 0.70 ‰ (Table 9.2; Fig 9.4). These results suggest that there were at least two trophic levels, with Heptageniidae and Blephariceridae as primary consumers and Buccinidae as a secondary consumer. Simuliidae and Hydropsychidae were in the middle, potentially being a third tropic level, with some taxa primary consumers and others secondary (Fig 9.5).

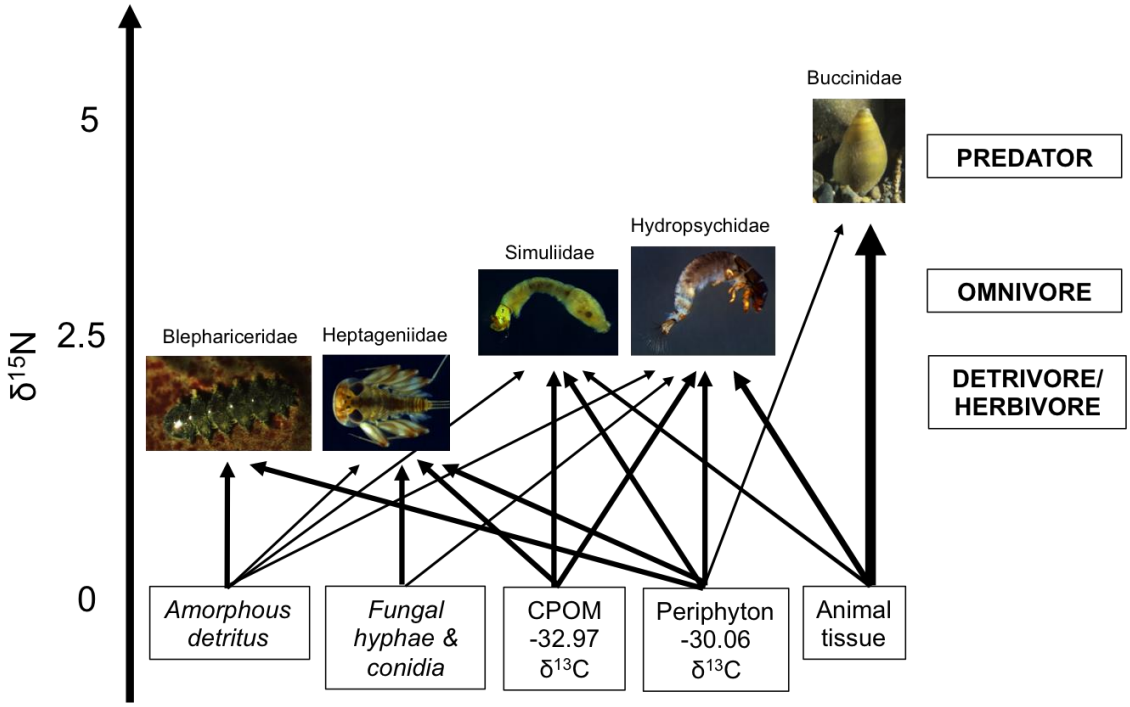


Figure 9.5. *Food web of the dominant macroinvertebrates present on rock based biotopes in Ulu Temburong National Park. Based on stomach contents analysis and stable isotope analysis. Line thickness represents contributions of the sources.*

9.4 Discussion

Waterfalls have been ignored in many aspects of freshwater research (Rackemann *et al.*, 2013; Clayton and Pearson, 2016) and I am unaware of any previously published papers on waterfall food webs. Waterfalls are unique biotopes in tropical streams, lacking a hyporheic zone and a water column of sufficient depth for most fish (Clayton, 1995). Therefore the normally abundant herbivorous fish and shrimp are absent, leaving highly specialized macroinvertebrates to dominate the waterfalls (Clayton, 1995; Hart and Finelli, 1999). This study using both SIA and GCA has established a trophic structure of waterfalls in pristine catchments in northern Borneo showing there are two, and potentially three, trophic levels. These include the detritivore/herbivore (Heptageniidae and Blephariceridae), omnivore (Simuliidae and Hydropsychidae) and predator (Buccinidae; Fig 9.4). There was some variation in carbon and nitrogen isotopic values of macroinvertebrates among the waterfalls, which is likely linked to variations in functional habitats including both quantity and quality. Given that this is amongst one of the few studies on waterfall trophic structure (Rackemann *et al.*, 2013; Clayton and Pearson, 2016), further research relating functional

habitats and waterfall geomorphology to the trophic ecology of waterfall resident taxa would be required to elucidate these patterns.

The isotopic values ($\delta^{13}C$ and $\delta^{15}N$) of basal food sources were distinct for periphyton and leaf litter (Table 9.2), making it possible to use them as indictors to distinguish between autochthonous and allochthonous basal food resources. The $\delta^{13}C$ values for leaf litter averaged -32.97 ‰, which was slightly lower compared to other values reported from the tropics. For example, Huang *et al* (2007) had CPOM from streams in Taiwan with an average of -30.3 ‰ and values from Hong Kong averaged -31.4 ‰ (Mantel *et al.*, 2004). This range in $\delta^{13}C$ values can be likely attributed to the high tree diversity in tropical rainforests, for example in Ulu Temburong national park there are approximately 250 species of trees (Edwards 1994). This study sampled random leaf litter on waterfalls; not focusing on a certain species therefore variation in $\delta^{13}C$ values when compared to other studies is understandable.

The average $\delta^{13}C$ value of periphyton was -30.06‰ (Table 9.2). This was more depleted compared to other studies; -22.96‰ (Puerto Rico, March and Pringle, 2003), -25.4‰ (Hong Kong, Lau *et al.*, 2009b) and -21‰ (Guadeloupe, Coat *et al.*, 2009). However, depleted $\delta^{13}C$ values of filamentous algae have been reported, such as -28.3‰ in Hong Kong (Mantel *et al.*, 2004). The difference in periphyton $\delta^{13}C$ values reported in the literature compared to periphyton in our study could be a result of samples being taken from pools and riffles, biotopes with different environmental conditions compared to waterfalls. The $\delta^{13}C$ values of periphyton can be affected by several factors including the availability of dissolved inorganic carbon, taxonomic composition of the periphyton, water velocity, growth rate, and periphyton biomass (Hill and Middleton, 2006). The average $\delta^{15}N$ for periphyton (-1.29 ‰) was higher than in leaf litter (-0.91 ‰; Table 9.2). Not surprising as periphyton scrubs contain a complex mix of bacteria, algae, fungi, protozoa and can include animal tissue, thus increasing the $\delta^{15}N$ value (Wetzel, 2001; March and Pringle, 2003).

This study was conducted during the dry season (i.e., June- August 2014) when there are high rates of leaf litter fall (Edwards, 1994; Pendry and Proctor, 1996). Leaves were therefore expected on all waterfalls during this study. However, during the rest of the year when there is less leaf litter fall and faster river flows, it would be predicted there would be less leaf litter on the waterfalls. This study showed the importance of periphyton as a food source. Waterfalls are often situated under a canopy gap due to the wide plunge pools directly

below the waterfall, which increase the channel width. Therefore, unlike other headwater tropical biotopes, waterfalls are less likely to be light limited. In addition to leaf litter and periphyton food sources, some macroinvertebrate guts contained fungal hyphae and conidia. It has long been acknowledged that microbial conditioning of leaf litter increases nutritional value and palatability for macroinvertebrates (Petersen and Cummins, 1974). In the tropics bacteria and fungi has been suggested to play a more important role in leaf litter decomposition due to the higher lignin content (Irons *et al.,* 1994; Wallace *et al.*, 1997) and in the streams of Ulu Temburong it is not uncommon to see leaf litter covered in fungi.

Both Heptageniidae and Blephariceridae are defined as herbivorous grazers in the literature (Tonnoir 1930; Alverson *et al.,* 2001; Ghee, 2004). However, in this study Blephariceridae had $\delta^{13}C$ values matching both periphyton and leaf litter. It's possible for the Blephariceridae to graze the periphyton and fungi that have colonized the leaf surface and as a consequence consume the leaf litter (Petersen and Cummins, 1974). The $\delta^{15}N$ values of Heptageniidae and Blephariceridae generally stayed under 2.5 ‰ implying these taxa are herbivorous (Table 9.2), with the GCA supporting these results with no animal tissue present.

In the literature Simuliidae and Hydropsychidae have been classed as filter feeders therefore it is surprising that periphyton was the abundant food source (Cummins, 1973; Cummins and Klug 1979; Fig 9.4). However, these functional feeding group classifications are based on taxa living in temperate streams and there is a growing number of studies that suggest tropical taxa are more dependent on periphyton than leaf litter or detritus (March and Pringle, 2003; Brito *et al.*, 2006; Lau *et al.,* 2009a). For example, Brito *et al.,* (2006) found Hydropsychidae in a Brazilian stream, with $\delta^{13}C$ values matching riverweed and microalgae. The low digestibility of tropical leaf litter as suggested by the C: N values in this study may explain why periphyton is a more popular food source (Table 9.2; Benstead, 1996). Wooton (1977) argues that Simuliidae have been known to eat algae and will essentially ingest any particle from 0.5 μm to a maximum that depends on their mouth size (Burton, 1973). In addition, it is possible the water column contains many fragments of periphyton due to the frequent scouring flows. In Ulu Temburong National Park convective rainfall causes river and stream levels to fluctuate, this can occur daily in the wet season, with the power of the water scouring out habitats including periphyton (Sheldon, 2011). These consistent fast flows

may have also influenced macroinvertebrate feeding behaviour, causing taxa to scrape algae from rocks instead of filtering particles from the fast flowing water.

The SIA results showed that Simuliidae and Hydropsychidae had higher $\delta^{15}N$ values compared to herbivorous grazers (Heptageniidae and Blephariceridae; Table 9.2). The $\delta^{15}N$ values of consumers tend to be 2-5 ‰ higher than that of their diets (Hobson and Clark, 1992; Bearhop *et al.,* 2002), therefore our results suggest that Simuliidae and Hydropsychidae on the waterfalls were omnivorous. In the literature Simuliidae have been known to consume Chironomidae (Serra-Tosio, 1967) and some studies have regarded Hydropsychidae as omnivorous and even predacious (Fuller and Mackay, 1980; Allan and Castillo, 1995). The GCA support the SIA with animal tissue present in the guts of both Simuliidae and Hydropsychidae. This study is the first known stable isotope analysis on Buccinidae snail in a Bornean stream, with the literature mainly focusing on its taxonomy and geographic distribution (Polgar *et al.*, 2015). Our results show that the snail had little overlap with $\delta^{13}C$ values for leaf litter or periphyton (Table 9.2) and the $\delta^{15}N$ values confirm reports in the literature that it is predacious (Coelho *et al.*, 2013).

Some taxa showed high variability of $\delta^{13}C$ and $\delta^{15}N$ values (Table 9.2). This variability could be due to the coarse level of identification to family level. Identification to genus or species level may yield greater information, with species potentially having different life histories and food preferences. However, this level of resolution is difficult in Borneo with macroinvertebrates still mostly undescribed (Yule and Yong, 2004; Jacobsen *et al.,* 2008). Variability in dietary composition could also be a result of substrate complexity along with food availability and quantity, which was not formally investigated. In our study the waterfall's geomorphic complexity varied from smooth bedrock to substrate covered in numerous ridges and holes promoting the formation of a range of functional habitats defined by a mix of leaf litter, wood debris, moss and periphyton to accumulate. Although these functional habitats were not quantitatively recorded in this study, visual observations found higher levels of wood debris and leaf litter on some waterfalls, particularly ones with complex substrate. Waterfall complexity may also influence taxa abundance on the waterfall and therefore number of prey for the predators. For example, Buccinidae $\delta^{15}N$ value was lowest (4.35 ‰) at waterfall 9 which had smooth substrate. While, the highest level of $\delta^{15}N$ (6.63 ‰) was at waterfall 1 which had heterogeneous substrate. However, further studies are

required to investigate if these changes in waterfall complexity influence macroinvertebrate feeding behaviour and trophic level.

While the food web is mostly resolved it appears there are likely some unknown food sources. The first was a source with more negative $\delta^{13}C$ values (less than -33‰) that matched Blephariceridae and Heptageniidae at waterfall 10 (Fig 9.4). Reviewing other tropical isotopic studies (including March and Pringle, 2003; Brito *et al.*, 2006; Coat *et al.*, 2009; Lau *et al.,* 2009b) few food sources matched such low $\delta^{13}C$ values. However, Huang *et al.,* (2007) did sample red algae, which had depleted ^{13}C with values around -37.4‰. Although this does not exactly match the $\delta^{13}C$ values of Blephariceridae and Heptageniidae it could suggest that the study waterfalls do harbour some types of algae with very depleted $\delta^{13}C$ values. The second 'unknown' food source had a $\delta^{13}C$ value of -27‰ and matched the $\delta^{13}C$ value for Heptageniidae at waterfall 7, Simuliidae at waterfall 11 and Hydropsychidae at waterfall 8 and 11. These values match values of dissolved organic matter and Fine Particulate organic matter (FPOM) from other studies, 28.6‰ on the Island of Guadeloupe (Coat *et al.*, 2009) and in a Brazilian stream FPOM had a mean $\delta^{13}C$ value of ~29‰ (Brito *et al.*, 2006).

9.5 Conclusions

Macroinvertebrate communities living on tropical waterfall are significantly under researched, with this study being the first known published paper to examine their trophic structure. Results from both SIA and GCA show two, and potentially three, trophic levels occurring including the herbivore (Heptageniidae and Blephariceridae), omnivore (Simuliidae and Hydropsychidae) and predator (Buccinidae). In agreement with tropical trophic structure studies on pools and riffles, the basal food resource on the waterfalls were found to be algae based. However, this study does suggest that despite the scouring velocities, waterfalls can retain functional habitats, with leaf litter being assimilated. The degree of functional habitat retention appears to be dependent on substrate heterogeneity and complexity. Quantitative analysis of substrate complexity is necessary to understand if the macroinvertebrate isotopic variation is related to waterfall complexity. In addition, further research is required to identify the unknown food sources.

Chapter 10: Summary

10.1 Overview of Research Aims and Questions

It is important to develop an understanding of the basic dynamics of pristine tropical stream systems in terms of eco-hydromorphology to assist with sensible conservation management and for robust ecosystem monitoring. There has been very little published data regarding tropical streams in Borneo (however see: Dolný *et al.,* 2011; Iwata *et al.,* 2003) and prior to this study there had been no extensive surveys of the benthic communities and ecosystem functions of the streams and rivers of Ulu Temburong National Park. The overall aim of this thesis was therefore to develop an understanding of the basic dynamics of tropical stream systems in terms of eco-hydromorphology, with an emphasis on benthic macroinvertebrates in the context of temperate stream classification models and theories. In the following section I review the general findings from each of the data chapters with reference to each of my initial research questions (see Chapter 2).

Chapter Four: This PhD thesis started with an expedition organised by the International Consortium of Universities for the Study of Biodiversity and the Environment (iCUBE) to Bukit Pagon (1850m), Brunei's highest mountain in 2012. The main goal of the expedition was to conduct a systematic study of the environment and biodiversity of high altitude primary rainforest in Brunei. The first data chapter of this thesis sought to answer ***are biotopes a useful classification tool for examining macroinvertebrate biodiversity in tropical streams (QC4)?*** From this study, 43 macroinvertebrate taxa were recorded in two remote, tropical streams in the Bukit Pagon catchment using the biotope classification tool. Biodiversity was similar between the study streams but there were differences among biotopes (pools, riffles, runs) with the lowest diversity occurring in fast-flowing biotopes. Community structure also varied among the biotopes. The dendrogram of macroinvertebrate abundance revealed a 0.8 dissimilarity between the fast and slow biotopes. Several taxa were found in multiple biotopes, which is likely linked to the occurrence of moss and leaf litter. Macroinvertebrate size structure distribution between the fast and slow biotopes was statistically different. Findings from this initial study gave enough evidence to suggest that biotopes may be an appropriate scale to

investigate macroinvertebrate biodiversity in the tropical streams of Temburong and thus became a suitable scale of study for the rest of the thesis.

Chapter Five: This data chapter was written as a short communication of the distribution and diversity of macroinvertebrates in the two main rivers that flow through Ulu Temburong National Park; Sungai Temburong and its tributary Sunagi Belalong. Due to the hydrology of the two rivers, with strong currents and flashy flows, the rivers are generally too dangerous to sample (Fig. 10.1). However, during a dry spell in 2014 it was possible to take the first recorded, systematic survey of the rivers to assess macroinvertebrate community structure and biodiversity of the pools. ***This chapter answered the research question (QC5) of whether macroinvertebrate diversity is similar between the two main river's of Ulu Temburong National Park, Sungai Temburong and Sungai Belalong?*** In both Temburong and Belalong, 6 pools were sampled once during the dry season of 2014. Sampling sites on Temburong were split with 3 pools upstream of the confluence with Belalong and 3 pools downstream of the confluence. Overall in the two rivers, 1,184 individuals were collected representing 55 genera from 36 families and 8 orders. Among the orders, Ephemeroptera had the highest abundance with Hemiptera and Lepidoptera having the lowest. There was no significant difference in macroinvertebrate richness, abundance or biomass between the two rivers and community analysis found taxa communities to be similar between the two rivers. However, there was a significant difference in abundance between the pools upstream and downstream of the confluence on Temburong. Although this study only provided preliminary results of benthic biodiversity in the pools of Temburong and Belalong, these rivers connect up the main study streams of this thesis and are therefore of interest to investigate the overall river continuum. Due to the lack of research on tropical rivers, it is hoped that this information will help increase the knowledge base for understanding the benthic ecology and encourage further study.

Figure 10.1 *(Originally Figure 5.2) Photo of Sungai Belalong outside KBFSC. Photo on the left taken at 17.45 when the rain started and photo on the right taken at 20:00*

Chapter Six: The hydraulic power of Sungai Temburong and Belalong was overwhelming to observe during fieldwork, with the two rivers levels 'bouncing up and down' almost daily (Fig. 10.1). During storm events I would watch the main river expand in size and with increasing discharge causing 'hydraulic damming' on the tributaries, with the main river preventing tributaries from flowing into the main river. During large storm events the main river would even flow up the tributaries, only stopping when reaching a waterfall or steep cascade. Confluences of rivers and their tributaries are complex, hydraulic habitats, which have been generally overlooked by river scientists. To date most research on these unique habitats has focused on the effect of tributaries on the main rivers; by contrast there have been very few studies on how the main rivers affect their tributaries. ***Therefore the aim of this chapter was to answer research question QC6 of whether organic matter (including leaf litter and wood debris) and levels of periphyton increased at the confluence? And whether this created 'biodiversity hotspots' for macroinvertebrates at the confluences?*** In 11 confluence zones, functional habitats and macroinvetebrates were sampled at the river margin (R), river-stream interface (I) and an upstream pool (U) that was above a high gradient riffle or waterfall where the main river only interacts during a significant storm event. At each location, leaf litter, wood debris, periphyton and macroinvertebrates were collected. I found no significant differences for any of the organic matter or periphyton measurements. However, several macroinvertebrate biodiversity "hot spots" were identified at the river-stream interface. These results suggest that that higher macroinvertbrate biodiversity is linked to confluence hydrology (e.g., hydraulic damming and

damped scour from the main river) rather than the modified inputs and outputs of organic matter or variation in periphyton.

Chapter Seven: Results indicated that the "biotope" is an appropriate scale to link the community structure of macroinvertebrates to natural hydraulic and geomorphic conditions in streams in Ulu Temburong National Park. This chapter expanded on this theme. ***The aim of this chapter was to answer research question QC7 of whether biotopes are important, rather than streams or reaches, for the operational scale of tropical macroinvertebrate biodiversity?*** As stream macroinvertebrates are reliable indicators of environmental health, understanding their natural distribution in tropical streams is vital for successful management and conservation. This chapter characterised the effects of biotopes on macroinvertebrate community structure in three streams within Ulu Temburong National Park. Biotopes within these streams were categorised as either bedrock waterfalls and cascades, or mixed-substrate riffles and pools. In total, 119 taxa were collected from all sampled biotopes, but not all taxa were collected from each stream. Biotopes were statistically distinct in terms of taxonomic richness, but not mean individual density or average community biomass. There were differences in community structure between waterfalls, cascades, pools, and riffles (Fig 10.2). The survey suggested that pool and riffle biotopes were more vulnerable to scouring flows and had similar community structure, while waterfalls and cascades likely experienced lower shear stress during floods and had similar macroinvertebrate communities. Results from this chapter conclude that classification and mapping of macroinvertebrates with biotope theory in pristine, tropical streams is a useful framework for simplifying the many linkages between ecology, geomorphology, and hydrology.

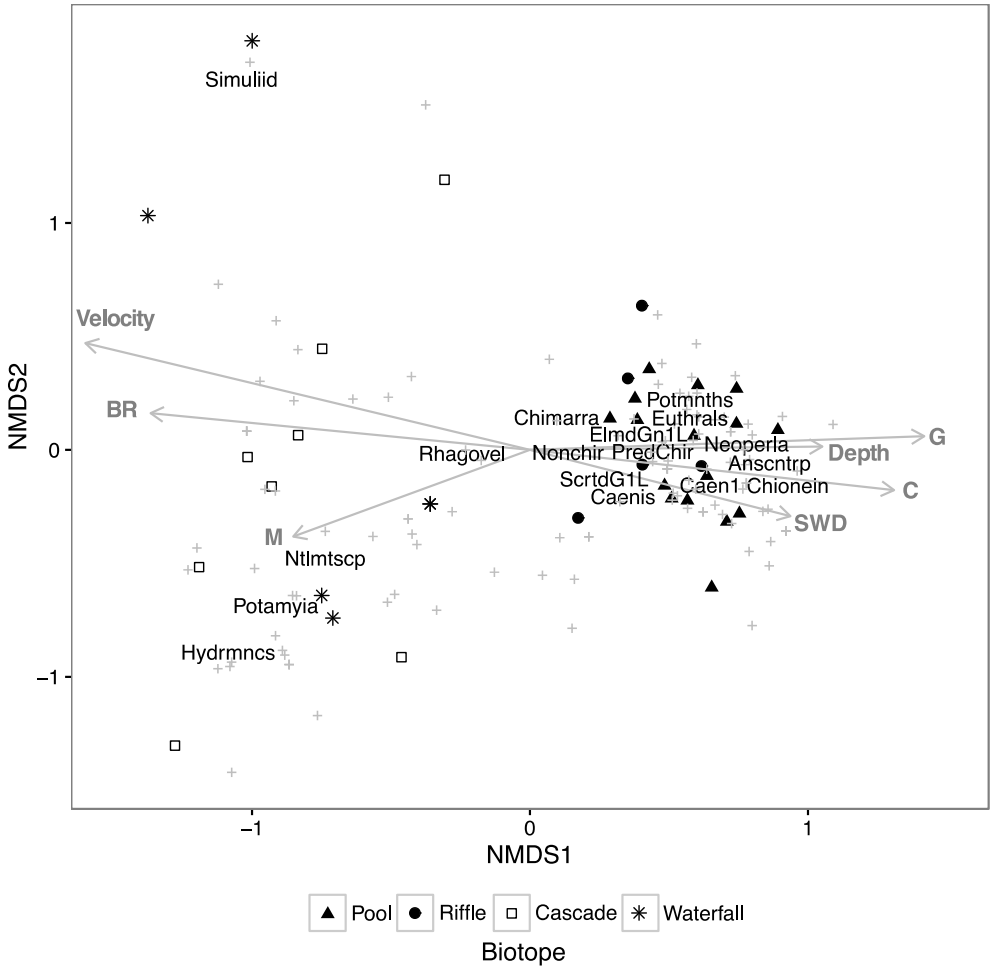


Figure 10.2. *(Originally Figure 7.5) Ordination of macroinvertebrate density among sampled biotopes. Environmental data were fitted to the ordination axis using the envfit function of the vegan package in R. The environmental factors displayed are those that are most strongly correlated with the taxa. This includes velocity, gravel (G), cobbles (C), and bedrock (BR; $p < 0.001$) as well as depth, small wood debris (SWD), and moss (M; $p < 0.05$). The biotopes are represented as symbols: pool, black triangle; riffle, black circle; cascade, white square; and waterfall, black star. List of taxa abbreviations are in appendix 7b (Stress = 0.16)*

Chapter Eight: The landscape of Ulu Temburong National Park changes over a relatively short distance from steep mountainous reaches at Bukit Pagon and Bukit Belalong to placid lowlands. This creates a geomorphic template for many waterfalls and cascades, which often partition streams into discrete zones. ***This chapter answered research question QC8 of to what extent do tropical waterfalls influence the distribution of aquatic communities? And will ecosystem functions (i.e. periphyton growth and leaf litter breakdown rates) differ between above- and below-waterfall pools?*** I observed higher fish densities in below-waterfall pools (0.24 fish m^{-2} versus 0.02 fish m^{-2} in above-waterfall pools) and higher relative shrimp abundance in above-waterfall pools (8

shrimp/pool versus <1 shrimp/pool in below-waterfall pools; Fig 10.3). However, macroinvertebrate densities (excluding shrimp) were similar among both pool types. Ambient periphyton standing stocks were higher in below-waterfall pools in 2013 (4.3 g m^{-2} versus 2.8 g m^{-2} in above-waterfall pools) and 2014 (4.8 g m^{-2} versus 3.4 g m^{-2} in above-waterfall pools), while periphyton growth rates varied from 0.05 to 0.26 g m^{-2} d^{-1} and were significantly higher in below-waterfall pools in 2014. Leaf litter decomposition rates (0.001 to 0.024 d^{-1}) did not differ between pool types, suggesting that neither shrimp nor fish densities had consistent impacts on this ecosystem function. Regardless, this research demonstrates the varied effects of biotic and abiotic factors on community structure and ecosystem function. These results highlight the importance of discontinuities, such as waterfalls, in tropical streams.

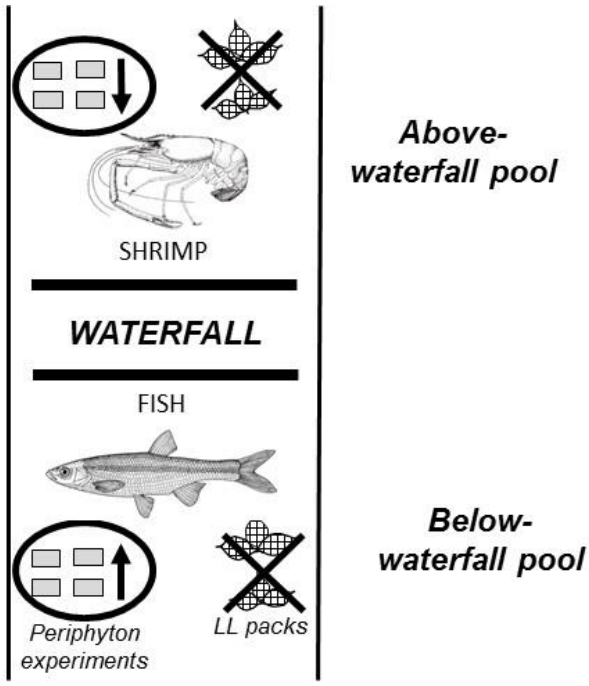


Figure 10.3. *Schematic displaying the influence of waterfalls on aquatic communities with fish dominated below-waterfall pools, while shrimp dominated above-waterfall pools. No difference in leaf litter (LL) decomposition between the above and below- waterfall pools but there was higher levels of periphyton in below- waterfall pools. Image of fish Susan Laurie Bourque © Canadian Museum of Nature and image of the shrimp is from the Food and Agriculture Organization ©.*

Chapter Nine: Tropical waterfalls are unusual biotopes as they lack a hyporheic zone and a water column of sufficient depth for the normally abundant shrimp and herbivorous fish, leaving highly specialized macroinvertebrates to dominate the waterfalls. This study used stable isotope analysis (SIA; $\delta^{13}C$ and $\delta^{15}N$ of leaf litter and periphyton) and gut contents analysis (GCA) to investigate the trophic structure of the abundant macroinvertebrates living on waterfalls including Heptageniidae, Blephariceridae, Simuliidae, Hydropsychidae, and Buccinidae. ***This chapter answered research question QC9, what is the main source of food for tropical macroinvertebrates that live on waterfalls?*** The $\delta^{15}N$ values ranging from -1.9‰ to 5.5‰ show there are herbivores (Heptageniidae and Blephariceridae), omnivores (Simuliidae and Hydropsychidae) and predators (Buccinidae) living on the waterfalls in Ulu Temburong. Apart from Buccinidae, all the taxa had $\delta^{13}C$ signatures ranging from 33‰ to 26‰, which mostly matched periphyton and leaf litter. GCA was useful to support the SIA results showing Simuliidae and Hydropsychidae guts contained animal tissue, while Heptageniidae and Hydropsychidae guts contained fungal hyphae and conidia. To our knowledge this paper is the only published study to examine their trophic structure. Results show the high dependence on periphyton is similar to other tropical biotopes, however results show that leaf litter and other food types are also important food sources. Combining both SIA and GCA I have established the trophic structure of tropical waterfalls showing there are two, and potentially three, trophic levels (Fig 10.4). Further research is required to expand on the number of food sources for the stable isotope analysis to identify the two 'unknown' food sources. In addition more quantitative analysis of substrate complexity work is necessary to understand if the macroinvertebrate isotopic variation is related to waterfall complexity and abundance of functional habitats.

In addition to the general research questions this thesis has produced some first recordings of aquatic insects in Brunei: *Compsoneuriella* sp. (Ephemeroptera: Baetidae); *Pelthydrus elongatulus* (Coleoptera: Hydrophilidae; Schonmann 1995), *Schinostethus* sp. (Coleoptera: Psephenidae), *Dryopomorphus memei* (Coleoptera: Elmidae; Čiampor *et al.,* 2012, Sartori and Gattolliat, *personal communication*, 2014, Manfred, *personal communication*, 2014).

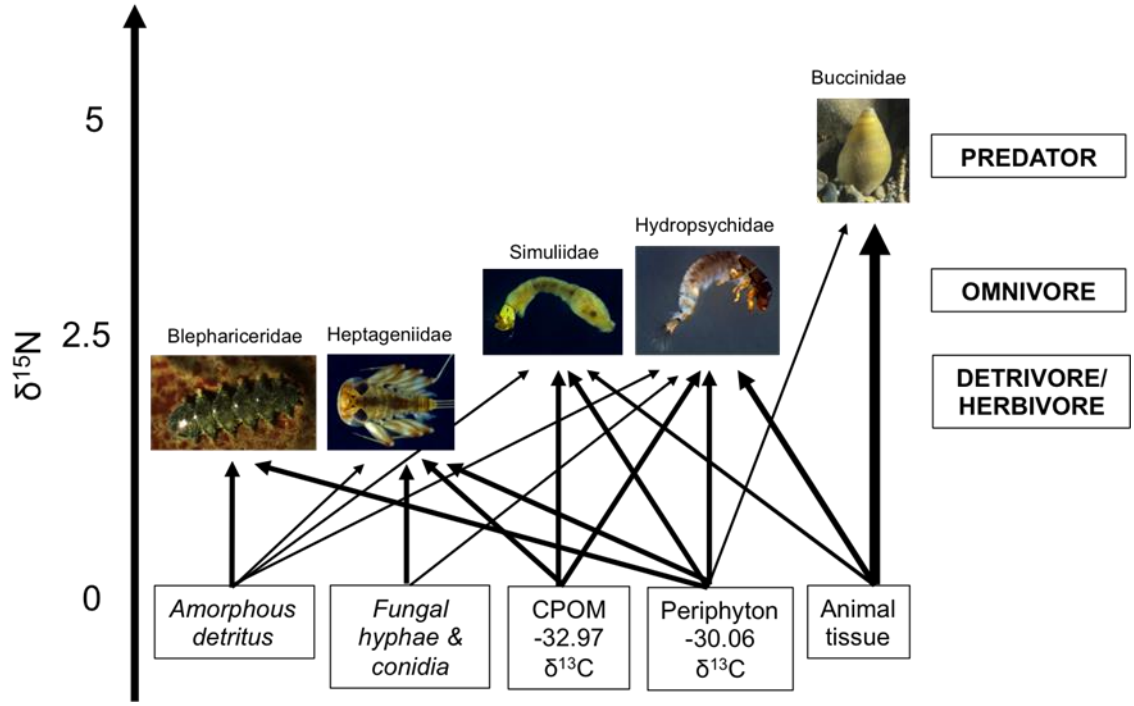


Figure 10.4. *(Originally Figure 9.4) Food web of the dominant macroinvertebrates present on rock based biotopes in Ulu Temburong National Park. Based on stomach contents analysis and stable isotope analysis. Line thickness represents contributions of the sources.*

10.2 Recommendations for Future Work

The linkages between the ecology, geomorphology and hydrology in tropical streams are complex. This thesis has made a solid contribution to understanding some of these linkages but nonetheless following on from this work I propose a number of future research investigations:

- Quantify the frequent hydraulic changes and associated disturbance which occur within the rivers, streams and confluence zones with environmental sensors. A long-term data set was not possible during this thesis, however environmental sensors have now reduced in price and they would provide much needed information on river and stream discharge in Ulu Temburong catchment.
- The frequent hydraulic disturbances cause substrate and functional habitats to shift and change on a daily basis. Monitoring over a period of time may assist in further understanding how this frequent habitat disturbance influences benthic communities and functional habitats. For example, in tropical streams do the more transient

functional habitats (e.g. leaf litter and small wood debris) have less value to benthic communities compared to attached functional habitats (e.g. moss, trailing roots)?

- Size structure of macroinvertebrates among the biotopes. This study has demonstrated that the bedrock biotopes (cascades and waterfalls) and mixed substrate biotopes (pools and riffles), harboured different communities, and levels of taxa richness. Do the more consistent environmental conditions of waterfalls and cascades, in comparison to the mixed-substrate biotopes (pools and riffles), have an influence on their size? This is an important consideration for tropical stream productivity.
- Macroinvertebrate dependence on periphyton on waterfalls is similar to other tropical biotopes, however results show that leaf litter and other food types are also important food sources. Further research is required to expand on the number of food sources found from stable isotope analysis. For example in this study there were two 'unknown' food sources. In addition more quantitative analysis of substrate complexity work is necessary to understand if the macroinvertebrate isotopic variation is related to waterfall complexity and the subsequent increased abundance of functional habitats.
- In addition there are a range of topics and questions linked to:
    - Climate change affects in a catchment free from land-use change
    - Systematic collections of inverts to provide a catalogue of resident taxa
    - Fish biodiversity
    - System productivity

10.3 Concluding Remarks

This PhD thesis has focused on the basic dynamics of pristine tropical stream systems in Ulu Temburong National Park. This region was used as a case study owing to its pristine rainforests and stream systems, which have been little studied and yet vitally important for regional conservation. The high levels of land use change in surrounding areas of northern Borneo and within S.E Asia as a whole, creates a vital requirement to develop an understanding of the basic dynamics of tropical stream systems in terms of eco-hydromorphology. This thesis has focused on benthic macroinvertebrates and used temperate stream classification models and theories to improve understanding about eco-

hydrogeomorphological in tropical stream systems. It is hoped that this knowledge will assist future successful management and conservation projects.

References

Abell, R., Thieme, M. L., Revenga, C., Bryer, M., Kottelat, M., Bogutskaya, N., Coad, B., Mandrak, N., Balderas, S. C. and Bussing, W. (2008) Freshwater ecoregions of the world: a new map of biogeographic units for freshwater biodiversity conservation. *BioScience,* 58(5), 403-414.

Adams, W. M., Perrow, M. R. and Carpenter, A. (2004) Conservatives and champions: river managers and the river restoration discourse in the United Kingdom. *Environment and Planning A,* 36(11), 1929-1942.

Ahmad Sah H. H., Grafe T. U, Dornburg, A., McLeod D. S., Bauer A. M., Wahab R. A, Grismer, L., and Watkins-Colwell G. W. (2016) The Amphibians, Reptiles and Fishes of the 2012 Bukit Pagon Expedition, *Brunei Darussalam Bulletin of the Peabody Museum of Natural History,* 57, 97-114.

Allan, J. D. and Castillo, M. M. (1995) *Stream ecology*. Springer, Netherlands

Alverson, A.J., Courtney, G.W. and Luttenton, M.R. (2001) Niche overlap of sympatric Blephariceraa larvae (Diptera: Blephariceridae) from the southern Appalachian Mountains. *Journal of the North American Benthological Society*, *20*(4), pp.564-581.

Amoros, C., Gibert, J., and Greenwood, M. T. (1996) 'Interactions between units of the fluvial hydrosystem', in Petts, G.E. and Amoros, C. (Eds), Fluvial Hydrosystems. Chapman and Hall, London. pp. 184–210.

Anderson, K. E., Paul, A. J., McCauley, E., Jackson, L. J., Post, J. R. and Nisbet, R. M. (2006) Instream flow needs in streams and rivers: the importance of understanding ecological dynamics. *Frontiers in Ecology and the Environment,* 4(6), 309-318.

Armitage, P. (1989) The application of a classification and prediction technique based on macroinvertebrates to assess the effects of river regulation. *In:* J. A. Gore & G. E. Petts (Eds.) *Alternatives in Regulated River Management*. CRC Press Incorporated, Boca Raton, Florida.

Armitage, P. D., Pinder, L. C., Cranston, P. (2012) The Chironomidae: biology and ecology of non-biting midges. Springer Science & Business Media, Netherlands.

Arsuffi, T. L. and Suberkropp, K. (1985) Selective Feeding by Stream Caddisfly (Trichoptera) Detritivores on Leaves with Fungal-Colonized Patches. *Oikos,* 45(1), 50-58.

Bass, D., (2007) Freshwater macroinvertebrates and their habitats in Dominica. Living World, Journal of the Trinidad and Tobago Naturalists Club, 21-30.

Bearhop, S., Adams, C. E., Waldron, S., Fuller, R. A. and Macleod, H. (2004) Determining trophic niche width: a novel approach using stable isotope analysis. *Journal of Animal Ecology,* 73(5), 1007-1012.

Beckmann, M. C., Schöll, F. and Matthaei, C. D. (2005) Effects of increased flow in the main stem of the River Rhine on the invertebrate communities of its tributaries. *Freshwater Biology,* 50(1), 10-26.

Begon, M., Townsend, C. R. and Harper, J. L. (2006) *Ecology: from individuals to ecosystems*. London: Wiley & Sons.

Beisel, J. N., Usseglio-Polatera, P. and. Moreteau. J. C (2000) The spatial heterogeneity of a river bottom: a key factor determining macroinvertebrate communities. *In:* M. Jungwirth, S. Muhar, and S. Schmutz, (Eds). *Assessing the Ecological Integrity of Running Waters*. Springer, Netherlands.

Benda, L., Poff, N. L., Miller, D., Dunne, T., Reeves, G., Pess, G. and Pollock, M. (2004) The network dynamics hypothesis: how channel networks structure riverine habitats. *BioScience,* 54(5), 413-427.

Benke, A. C., A. D. Huryn, L. A. Smock, and J. B. Wallace. (1999) Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. *Journal of the North American Benthological Society*. 18: 308-343.

Benstead, J. P. (1996) Macroinvertebrates and the Processing of Leaf Litter in a Tropical Stream. *Biotropica,* 28(3), 367-375.

Best, J. L. (1988) Sediment transport and bed morphology at river channel confluences. *Sedimentology,* 35(3), 481-498.

Biggs, B., Stevenson, R. and Lowe, R. (1998) A habitat matrix conceptual model for stream periphyton. *Archiv für Hydrobiologie,* 143(1), 21-56.

Biggs, T., Dunne, T. and Martinelli, L. (2005) Natural controls and human impacts on stream nutrient concentrations in a deforested region of the Brazilian Amazon basin. *Biogeochemistry,* 68(2), 227-257.

Bishop, J. E. (1973). *Limnology of a small Malayan river Sungai Gombak*. Dr. W. Junk Publishers: The Hague.

Bisson, P. A., Nielsen, J. L., Palmason, R. A. and Grove, L. E. (1982) A system of naming habitat types in small streams, with examples of habitat utilization by salmonids during low streamflow. *Acquisition and utilization of aquatic habitat inventory information. American Fisheries Society, Western Division, Bethesda, Maryland*, 62-73.

Blanco-Belmonte L. (2006) Variación espacial de la comunidad de macro-invertebrados en tres fases hidrológicas en el Bajo Caura (Venezuela). Magíster Thesis, Universidad Nacional Experimental de Guayana, Ciudad Guayana.

Brierley, G. J. and Fryirs, K. A. (2008) *Geomorphology and river management: applications of the river styles framework*. John Wiley & Sons. FROM: Rosales, J., Blanco-Belmonte, L., Bradley, C. (2008) Hydrogeomorphological and ecological interactions in tropical floodplains: the significance of confluence zones in the Orinoco basin, Venezuela. In: Wood, P.J., Hannah, D.M., Sadler, J.P. (Eds.), *Hydroecology and Ecohydrology: Past, Present and Future*. J. Wiley, Chichester, 295-316.

Bojsen, B. H. and D. Jacobsen. (2003). Effects of deforestation on macroinvertebrate diversity and assemblage structure in Ecuadorian Amazon streams. *Archiv für Hydrobiologie*, 158:317-342.

Bond, B. (2003) Hydrology and ecology meet-and the meeting is good. *Hydrological processes,* 17(10), 2087-2089.

Bond, N. R. and Downes, B. (2000) Flow-related disturbance in streams: an experimental test of the role of rock movement in reducing macroinvertebrate population densities. *Marine and Freshwater Research,* 51(4), 333-337.

Borcard, D., F. Gillet, and P. Legendre. (2012). *Numerical Ecology with R*. Springer, New York.

Boulton, A. J., Boyero, L., Covich, A. P., Dobson, M., Lake, S. and Pearson, R. (2008) Are Tropical Streams Ecologically Different from Temperate Streams? In: Dudgeon D (Ed) *Tropical Stream Ecology*, Academic Press, San Diego.

Boyero L, Bailey R. C. (2001). Organization of macroinvertebrate communities at a hierarchy of spatial scales in a tropical stream. *Hydrobiologia* 464:219-225.

Boyero, L., Ramirez, A., Dudgeon, D. and Pearson, R. G. (2009) Are tropical streams really different? *Journal of the North American Benthological Society,* 28(2), 397-403.

Brierley, G. J. and Fryirs, K. A. (2008) *Geomorphology and river management: applications of the river styles framework*. John Wiley & Sons, Oxford

Brierley, G., Fryirs, K., Cullum, C., Tadaki, M., Huang, H. Q. and Blue, B. (2013) Reading the landscape Integrating the theory and practice of geomorphology to develop place-based understandings of river systems. *Progress in Physical Geography*, *37*(5), 601-621.

Brierley, G.J. and Fryirs, K.A. (2008) Move towards an era of river repair In: Brierley, G.J. and Fryirs, K.A. and Hobbs, R. J. *(2008) River Futures: An Integrative Scientific Approach to River Repair.* Island Press, Washington DC.

Brito, E., Moulton, T., De Souza, M. and Bunn, S. (2006) Stable isotope analysis indicates microalgae as the predominant food source of fauna in a coastal forest stream, south-east Brazil. *Austral ecology,* 31(5), 623-633.

Brown, T. G. and Hartman, G. F. (1988) Contribution of Seasonally Flooded Lands and Minor Tributaries to the Production of Coho Salmon in Carnation Creek, British Columbia. *Transactions of the American Fisheries Society,* 117(6), 546-551.

Bruns, D., Minshall, G., Cushing, C., Cummins, K., Brock, J. and Vannote, R. (1984) Tributaries as modifiers of the river continuum concept: analysis by polar ordination and regression models. *Archiv für Hydrobiologie,* 99(2), 208-220.

Bryan, J. E., Shearman, P. L., Asner, G. P., Knapp, D. E., Aoro, G. and Lokes, B. (2013) Extreme differences in forest degradation in Borneo: Comparing practices in Sarawak, Sabah, and Brunei. *PloS one,* 8(7).

Buendia, C., C. N Gibbins, D. Vericat, and R. J Batalla. (2014) Effects of flow and fine sediment dynamics on the turnover of stream invertebrate assemblages. *Ecohydrology*,7: 1105-1123.

Bunn, S. E. and Arthington, A. H. (2002) Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. *Environmental management,* 30(4), 492-507.

Burton, G. J. (1973) Feeding of Simulium Hargreavesi Gibbins Larvae on Oedegonium Algal Filaments in Ghana. *Journal of Medical Entomology,* 10(1), 101-106.

Chao, A., Gotelli, N. J., Hsieh, T., Sander, E. L., Ma, K., Colwell, R. K. and Ellison, A. M. (2014) Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. *Ecological Monographs,* 84(1), 45-67.

Cheshire, K. I. M., L. U. Z. Boyero, and R. G Pearson. (2005) Food webs in tropical Australian streams: shredders are not scarce. *Freshwater Biology* 50: 748-769.

Choy, S. C. & Chin, P. (1994) Fresh-water fishes from the headwaters of the Belalong-Temburong River System, Brunei-Darussalam, Borneo. *Raffles Bulletin of Zoology* 42, 757-774.

Choy, S. C. (1996) Distributional ecology of freshwater fishes in tropical rainforest streams of Borneo. In: Edwards, D. S., W. E. Booth & S. C. Choy (eds.), *Tropical Rainforest Research: Current Issues*. Kluwer Academic Publishers, Dordrecht.

Church, M. (2002) Geomorphic thresholds in riverine landscapes. *Freshwater Biology,* 47(4), 541-557.

Clarke, K. and Warwick, R. (2001) *Change in Marine Communities: An Approach to Statistical Analysis and Interpretation*. 2nd Edition ed. Plymouth: PRIMER-E Ltd.

Clarke, K. R. (1993) Non-parametric multivariate analysis of changes in community structure. *Australian Journal of Ecology* 18: 117–143.

Clayton, P. D. (1995). *The ecology of waterfalls in the Australian wet tropics*. James Cook University, Australia., James Cook University, Australia.

Clayton, P. D. and Pearson, R. G. (2016) Harsh habitats? Waterfalls and their faunal dynamics in tropical Australia. *Hydrobiologia,* 775(1), 123-137.

Clements, F. (1905) Research Methods in Ecology. Lincoln, NB: University Publishing Company. From: Hubbell, D. and Ryan, J. (2016) Scholarly Ecotones in the Information Landscape. *Landscapes: the Journal of the International Centre for Landscape and Language,* 7(1), 24.

Clifford, N. J., Harmar, O. P., Harvey, G. and Petts, G. E. (2006) Physical habitat, eco-hydraulics and river design: a review and re-evaluation of some popular concepts and methods. *Aquatic conservation: marine and freshwater ecosystems,* 16(4), 389-408.

Coat, S., Monti, D., Bouchon, C. and Lepoint, G. (2009) Trophic relationships in a tropical stream food web assessed by stable isotope analysis. *Freshwater Biology,* 54(5), 1028-1041.

Coelho, A. R., Dinis, M. T. and Reis, J. (2013) Effect of diet and stocking densities on life history traits of Clea helena (Philippi 1847) reared in captivity. *Journal of Aquaculture Research & Development,* 4 (5).

Corlett, R. T. (2009) *The ecology of tropical East Asia*. Oxford University Press: Oxford.

Coughlan, J. F., Pearson, R. G. and Boyero, L. (2010) Crayfish process leaf litter in tropical streams even when shredding insects are common. *Marine and Freshwater Research,* 61(5), 541-548.

Covich, A. P., Crowl, T. A. and Heartsill-Scalley, T. (2006) Effects of drought and hurricane disturbances on headwater distributions of palaemonid river shrimp (*Macrobrachium spp.*) in the Luquillo Mountains, Puerto Rico. *Journal of the North American Benthological Society* 25, 99-107.

Covich, A. P., Crowl, T. A., Hein, C. L., Townsend, M. J. and McDowell, W. H. (2009) Predator–prey interactions in river networks: comparing shrimp spatial refugia in two drainage basins. *Freshwater Biology,* 54(3), 450-465.

Covich, A. P., Palmer, M. A. and Crowl, T. A. (1999) The Role of Benthic Invertebrate Species in Freshwater Ecosystems: Zoobenthic species influence energy flows and nutrient cycling. *BioScience,* 49(2), 119-127.

Cranbrook, Earl of. (1993) Research and Management of the Batu Apoi Forest Reserve, Temburong, Brunei: The Universiti Brunei Darussalam/Royal Geographical Society Rainforest Project 1991/92. *Global Ecology and Biogeography Letters,* 3(4/6), 267-276.

Cranbrook, Earl of. & Edwards D. S. (1994) Belalong, a tropical rainforest. Sun Tree Publishing Ltd, Singapore.

Cranbrook, Earl of. (1993). Research and Management of the Batu Apoi Forest Reserve, Temburong, Brunei: The Universiti Brunei Darussalam/Royal Geographical Society Rainforest Project 1991/92. *Global Ecology and Biogeography Letters*, 3: 267-276.

Cressa, C. (1998). Community composition and structure of macroinvertebrates of the river Camurí Grande, Venezuela. *Verhandlungen-Internationale Vereinigung für theoretische und angewandte Limnologie* 26:1008-1011.

Crispo, E., Bentzen, P., Reznick, D. N., Kinnison, M. T. and Hendry, A. P. (2006) The relative influence of natural selection and geography on gene flow in guppies. *Molecular Ecology,* 15(1), 49-62.

Crowl, T. A., McDowell, W. H., Covich, A. P. and Johnson, S. L. (2001) Freshwater shrimp effects on detrital processing and nutrients in a tropical headwater stream. *Ecology,* 82(3), 775-783.

Crowl, T.A. and Covich, A.P., (1994). Responses of a freshwater shrimp to chemical and tactile stimuli from a large decapod predator. *Journal of the North American Benthological Society*, pp.291-298.

Cummins, K. W. (1973) Trophic relations of aquatic insects. *Annual review of entomology,* 18(1), 183-206.

Cummins, K. W. (1974) Structure and function of stream ecosystems. *BioScience,* 24(11), 631-641.

Cummins, K. W. and Klug, M. J. (1979) Feeding ecology of stream invertebrates. *Annual review of ecology and systematics,* 10, 147-172.

Dahl, F. (1908) Grundsaetze und grundbegriffe der biocoenotischen forshung. *Zoologischer Anzeiger,* 33, 349-353.

Davis, P. M., Bunn, S. and Hamilton, K. (2008) Primary Production in Tropical Streams and Rivers. *In:* D. Dudgeon (Ed.) *Tropical Stream Ecology*. London: Academic Press.

Death, R. G. (2002) Predicting Invertebrate Diversity from Disturbance Regimes in Forest Streams. *Oikos,* 97(1), 18-30.

Death, R. G. and Winterbourn, M. J. (1995) Diversity Patterns in Stream Benthic Invertebrate Communities: The Influence of Habitat Stability. *Ecology,* 76(5), 1446-1460.

Dial K P, Greene E, Irschick DJ. (2008) Allometry of behavior. *Trends in ecology & evolution* 23, 394-401.

Dias, M. S., Cornu, J.-F., Oberdorff, T., Lasso, C. A. and Tedesco, P. A. (2012) Natural fragmentation in river networks as a driver of speciation for freshwater fishes. *Ecography,* 36(6), 683-689.

Dolný, A., Bárta, D., Lhota, S. and Drozd, P. (2011) Dragonflies (Odonata) in the Bornean rain forest as indicators of changes in biodiversity resulting from forest modification and destruction. *Tropical Zoology,* 24(1).

Dudgeon D, Arthington A. H, Gessner M. O, Kawabata Z. L, Knowler D. J, Leveque C, Naiman R. J, Prieur-Richard A. H, Soto D, Stiassny M. L. J, Sullivan C. (2006)

Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological reviews* 81:163-182.

Dudgeon, D (1994) The influence of riparian vegetation on macroinvertebrate community structure and functional organization in six new Guinea streams. *Hydrobiologia* 294:65-85.

Dudgeon, D. (1991) An experimental study of the effects of predatory fish on macroinvertebrates in a Hong Kong stream. *Freshwater Biology* 25:321-330.

Dudgeon, D. (1993) The effects of spate-induced disturbance, predation and environmental complexity on macroinvertebrates in a tropical stream. *Freshwater Biology,* 30(2), 189-197.

Dudgeon, D. (1999) *Tropical Asian Streams: Zoobenthos, Ecology and Conservation*. Hong Kong University Press.

Dudgeon, D. (2006) The impacts of human disturbance on stream benthic invertebrates and their drift in North Sulawesi, Indonesia. *Freshwater Biology,* 51(9), 1710-1729.

Dudgeon, D. (2015) Freshwater Biology – sustaining excellence in a world of change. *Freshwater Biology* 60:1737-1739.

Dunham. A. E and Beaupre, S. J (1998) Ecological experiments, Scale, Phenology, Mechanism and the Illusion of Generality. *In*: Resetarits JR. W.J and J. Bernardo (Ed.) *Experimental Ecology, Issues and Perspectives*, Oxford University Press, New York.

Dykes, A. P. (1996) Analysis of factors contributing to the stability of steep hillslopes in the tropical rainforest of Temburong, Brunei Darussalam. *In:* D. S. Edwards, W. E. Booth & S. C. Choy Eds. *Tropical Rainforest Research — Current Issues*. Vol. 74: Springer Netherlands, 1996, 387-409.

Dykes, A. P. (1997) Rainfall interception from a lowland tropical rainforest in Brunei. *Journal of Hydrology,* 200(1–4), 260-279.

Dykes, A. P. (2000) Climatic patterns in a tropical rainforest in Brunei. *The Geographical Journal,* 166(1), 63-80.

Dykes, A. P. (1994) Landfrom Processes. *In:* Cranbrook, Earl of. & Edwards D. S. (Eds) Belalong, a tropical rainforest. Sun Tree Publishing Ltd, Singapore.

Ebersole, J. L., Liss, W. J. and Frissell, C. A. (2001) Relationship between stream temperature, thermal refugia and rainbow trout Oncorhynchus mykiss abundance in

arid-land streams in the northwestern United States. *Ecology of Freshwater Fish,* 10(1), 1-10.

Edwards, D. S. (1994) *Belalong, a tropical rainforest*. Sun Tree Publishing, Singapore.

Elliott, J. M. (2003) A comparative study of the dispersal of 10 species of stream invertebrates. *Freshwater Biology,* 48(9), 1652-1668.

El-Sabaawi, R. W., Marshall, M. C., Bassar, R. D., López-Sepulcre, A., Palkovacs, E. P. and Dalton, C. (2015) Assessing the effects of guppy life history evolution on nutrient recycling: from experiments to the field. *Freshwater Biology*, *60*(3), 590-601.

Endler, J. A. (1978) A predator's view of animal color patterns *Evolutionary biology*. Springer, 1978, 319-364.

Erman, D. C., and Erman. N. A. (1984) The response of stream macroinvertebrates to substrate size and heterogeneity. *Hydrobiologia* 108:75–82.

Everaert, G., De Neve, J., Boets, P., Dominguez-Granda, L., Mereta, S. T., Ambelu, A., Hoang, T. H., Goethals, P. L. and Thas, O. (2014) Comparison of the abiotic preferences of macroinvertebrates in tropical river basins. *PloS one,* 9(10).

FAO. (2014). *Brunei Darussalam.* Available: http://www.fao.org/nr/water/aquastat/countries_regions/brunei_darsm/index.stm. Last accessed 21st Jan 2014.

Feminella, J. W. and Hawkins C. P (1995) Interactions between Stream Herbivores and Periphyton: A Quantitative Analysis of past Experiments. *Journal of the North American Benthological Society* 14:465-509.

Fernandes, C. C., Podos, J. and Lundberg, J. G. (2004) Amazonian Ecology: Tributaries Enhance the Diversity of Electric Fishes. *Science,* 305(5692), 1960-1962.

Ferrington Jr, L. C. (2008) Global diversity of non-biting midges (Chironomidae; Insecta-Diptera) in freshwater. *Hydrobiologia* 595:447-455.

Fisher, S. G., Grimm, N. B., Marti, E. and Gomez, R. (1998) Hierarchy, spatial configuration, and nutrient cycling in a desert stream. *Australian Journal of Ecology,* 23(1), 41-52.

Flecker, A. S. (1992) Fish Trophic Guilds and the Stucture of a Tropical Stream: Weak Direct vs. Strong Indirect Effects. *Ecology,* 73(3), 927-940.

Flecker, A. S. and Feifarek, B. (1994) Disturbance and the temporal variability of invertebrate assemblages in two Andean streams. *Freshwater Biology,* 31(2), 131-142.

Flecker, A. S. and Taylor, B. W. (2004) Tropical fishes as biological bulldozers: Density effects on resource heterogeneity and species diversity. *Ecology,* 85(8), 2267-2278.

Forman, R. T. (1995) *Land mosaics: the ecology of landscapes and regions*. Cambridge university press, Cambridge.

Forti, M., Boulet, R., Melfi, A. and Neal, C. (2000) Hydrogeochemistry of a small catchment in Northeastern Amazonia: a comparison between natural with deforested parts of the catchment (Serra do Navio, Amapá State, Brazil). *Water, air, and soil pollution,* 118(3-4), 263-279.

Fox, L. R. (1977) Species Richness in Streams--An Alternative Mechanism. *The American Naturalist,* 111(981), 1017-1021.

Frissell, C. A. and Nawa, R. K. (1992) Incidence and Causes of Physical Failure of Artificial Habitat Structures in Streams of Western Oregon and Washington. *North American Journal of Fisheries Management,* 12(1), 182-197.

Fry, B. (1991) Stable Isotope Diagrams of Freshwater Food Webs. *Ecology,* 72(6), 2293-2297.

Fry, B. (2007) *Stable isotope ecology*. Springer Science & Business Media, United States.

Fuller, R. L. and Mackay, R. J. (1981) Effects of food quality on the growth of three Hydropsyche species (Trichoptera: Hydropsychidae). *Canadian Journal of Zoology,* 59(6), 1133-1140.

Furtado, J. I. (1969) Ecology of Malaysian odonates: Biotope and association of species. *Verh. Internat. Verein. Limnol,* 17, 863-887.

Garcia, E. A., Townsend S. A. and Douglas M. M. (2015) Context dependency of top-down and bottom-up effects in a Northern Australian tropical river. *Freshwater Science* 34:679-690.

Gessner, M. O., Chauvet, E. and Dobson, M. (1999) A perspective on leaf litter breakdown in streams. *Oikos*, 377-384.

Ghee, K. S. (2004) Insecta: Ephemeroptera. *In:* C. M. Yule & H.-S. Yong Eds. *Freshwater invertebrates of the Malaysian region*. Malaysia: Academy of Sciences Malaysia Kuala Lumpur, 2004.

Giller, P. S. and Malmqvist, B. (1998) *The biology of streams and rivers*. Oxford University Press, USA.

Gilliam, J. F., D. F. Fraser, D. F. & A. M. Sabat, (1989). Strong Effects of Foraging Minnows on a Stream Benthic Invertebrate Community. *Ecology* 70: 445-452.

Goodwin, C. N. (1999). *Fluvial Classification: Neanderthal Necessity or Needless Normalcy*. Paper presented at the Proceedings Wildland Hydrology American Water Resources Association, Bozeman, MT.

Gordon N. D, Finlayson B. L, McMahon T. A. (2004) *Stream hydrology: An introduction for ecologists*. John Wiley and Sons, New York

Gore, J. A. (1978) A technique for predicting in-stream flow requirements of benthic macroinvertebrates. *Freshwater Biology,* 8(2), 141-151.

Gore, J. A. and Judy Jr, R. D. (1981) Predictive Models of Benthic Macroinvertebrate Density for Use in Instream Flow Studies and Regulated Flow Management. *Canadian Journal of Fisheries and Aquatic Sciences,* 38(11), 1363-1370.

Gotelli, N. J. and Colwell, R. K., 12, (2011) Estimating species richness. *In:* A. E. Magurran & B. J. McGill Eds. *Biological diversity: frontiers in measurement and assessment*.: Oxford University Press, Oxford.

Gower J. C. (1971) A general coefficient of similarity and some of its properties. *Biometrics* 27:857-874.

Graça M. A, Ferreira W. R, Firmiano K, França J, Callisto M. (2015) Macroinvertebrate identity, not diversity, differed across patches differing in substrate particle size and leaf litter packs in low order, tropical Atlantic forest streams. *Limnetica* 34:29-40.

Greathouse, E. A. & C. M. Pringle (2006) Does the river continuum concept apply on a tropical island? Longitudinal variation in a Puerto Rican stream. *Canadian Journal of Fisheries and Aquatic Sciences* 63:134-152.

Greathouse, E. A., March, J. G. and Pringle, C. M. (2005) Recovery of a tropical stream after a harvest-related chlorine poisoning event. *Freshwater Biology,* 50(4), 603-615.

Gurnell, A.M., Corenblit, D., García de Jalón, D., González del Tánago, M., Grabowski, R.C., O'Hare, M.T. and Szewczyk, M., 2016. A conceptual model of vegetation–hydrogeomorphology interactions within river corridors. *River Research and Applications*, *32*(2), 142-163.

Hannah, D. M., Sadler, J. P. and Wood, P. J. (2007) Hydroecology and ecohydrology: a potential route forward? *Hydrological Processes,* 21(24), 3385-3390.

Hannah, D. M., Wood, P. J. and Sadler, J. P. (2004) Ecohydrology and hydroecology: A 'new paradigm'? *Hydrological Processes,* 18(17), 3439-3445.

Harper, D. M., Smith, C. D., Barham, P. J. and Howell, R. (1995) The ecological basis for the management of the natural river environment. *In:* D. M. Harper & A. J. D. Ferguson Eds. *The Ecological Basis for River Management*. Chichester: John Wiley, 1995, 219–238.

Harper, D., Smith, C., Barham, P. and Howell, R. (1995) The ecological basis for the management of the natural river environment. *The ecological basis for river management*, 219-238.

Hart, D. D. and Finelli, C. M. (1999) Physical-biological coupling in streams: the pervasive effects of flow on benthic organisms. *Annual Review of Ecology and Systematics*, 363-395.

Harvey, G. L. and Clifford, N. J. (2009) Microscale hydrodynamics and coherent flow structures in rivers: implications for the characterization of physical habitat. *River Research and Applications*, *25*(2) 160-180.

Harvey, G. L. Clifford, N. J. and Gurnell, A. M. (2008) Towards an ecologically meaningful classification of the flow biotope for river inventory, rehabilitation, design and appraisal purposes. *Journal of environmental management,* 88(4), 638-650.

Hawkins, C. P., Kershner, J. L., Bisson, P. A., Bryant, M. D., Decker, L. M., Gregory, S. V., McCullough, D. A., Overton, C. K., Reeves, G. H., Steedman, R. J. and Young, M.K. (1993) A hierarchical approach to classifying stream habitat features. *Fisheries*, *18*(6) 3-12.

Hein, C. L. and Crowl, T. A. (2010) Running the predator gauntlet: do freshwater shrimp (Atya lanipes) migrate above waterfalls to avoid fish predation? *Journal of the North American Benthological Society,* 29(2), 431-443.

Herrera J. K, and Rondón, J. (1985) Estudio fotogeomorfológico del Rió Orinoco y confl uencia con el Rió Apure. Sector Puerto Aycacucho, Rió Caura. In I Simposio Amazónico. M.I. Muñoz (ed), Boletín de Geología (Venezuela), Publicación Especial, 10: 361–372. FROM: Rosales, J., Blanco-Belmonte, L., Bradley, C. (2008) Hydrogeomorphological and ecological interactions in tropical floodplains: the significance of confluence zones in the Orinoco basin, Venezuela. In: Wood, P.J., Hannah, D.M., Sadler, J.P. (Eds.), *Hydroecology and Ecohydrology: Past, Present and Future*. J. Wiley, Chichester, 295-316.

Hershey, A. E. and Peterson, B. J. (1996) Stream food webs. *In:* Hauer, F.R. and Lamberti, G.A. (Eds) *Methods in Stream Ecology* Academic Press, London.

Hildrew, A. G. and Giller, P. S. (1994) Patchiness, species interactions and disturbance in the stream benthos. *In:* P. S. Giller, A. G. Hildrew & D. G. Raffaelli Eds. *Aquatic ecology: scale, pattern and process*. Oxford: Blackwell, 21-62.

Hill, M. O. (1979) TWINSPAN - A FORTRAN program for arranging multivariate data in an ordered two-way table by classification of the individuals and attributes , Ecology and Systematics, Cornell University, Ithaca, New York. .

Hill, W. R. and Middleton, R. G. (2006) Changes in carbon stable isotope ratios during periphyton development. *Limnology and Oceanography,* 51(5), 2360-2369.

Hill, W. R., Ryon, M. G. and Schilling, E. M. (1995) Light Limitation in a Stream Ecosystem: Responses by Primary Producers and Consumers. *Ecology,* 76(4), 1297-1309.

Hino, M. (1977) Eco-hydraulics, an attempt. Tech. Report no. 22, Department of Civil Engineering, Tokyo Institute of Hydrology, 29-59. (also presented at the XVIIth Congress of the International Association for Hydraulic Research, held in Baden-Banden in August 1977).

Ho, B. S. K. and Dudgeon, D. (2016) Are high densities of fishes and shrimp associated with top-down control of tropical benthic communities? A test in three Hong Kong streams. *Freshwater Biology,* 61(1), 57-68.

Hobson, K. A. and Clark, R. G. (1992) Assessing Avian Diets Using Stable Isotopes I: Turnover of ^{13}C in Tissues. *The Condor,* 94(1), 181-188.

Hohausová, E., Copp, G. H. and Jankovský, P. (2003) Movement of fish between a river and its backwater: diel activity and relation to environmental gradients. *Ecology of Freshwater Fish,* 12(2), 107-117.

Hora, S. L. (1922) Structural modifications in fish of mountain torrents. *Records of the Indian Museum,* 24, 31-61.

Hora, S. L. (1930) Ecology, Bionomics and Evolution of the Torrential Fauna, with Special Reference to the Organs of Attachment. *Philosophical Transactions of the Royal Society of London. Series B, Containing Papers of a Biological Character,* 218, 171-282.

Hsieh T. C, Ma, K. H, and Chao, A. (2016) iNEXT: An R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods in Ecology and Evolution* In revision.

Huang, I., Lin, Y., Chen, C. and Hsieh, H. (2007) Food web structure of a subtropical headwater stream. *Marine and Freshwater Research,* 58(7), 596-607.

Hubbell, D. and Ryan, J. (2016) Scholarly Ecotones in the Information Landscape. *Landscapes: the Journal of the International Centre for Landscape and Language,* 7(1), 24.

Huryn, A.D, Riley, R.H., Young, R.G, Arbuckle, C.J. and Peacock, K. (2002). Natural-abundance stable C and N isotopes indicate weak upstream-downstream linkage of food webs in a grassland river. *Archiv für Hydrobiologie*, *153*(2), 177-196.

Hynes, H. B. N. (1971) *The biology of polluted waters*. Liverpool, UK.: Liverpool University Press.

Irons, J. G., Oswood, M. W., Stout, R. J. and Pringle, C. M. (1994) Latitudinal patterns in leaf litter breakdown: is temperature really important? *Freshwater Biology,* 32(2), 401-411.

Iwata, T., Nakano, S. and Inoue, M. (2003) Impacts of past riparian deforestation on stream communities in a tropical rain forest in Borneo. *Ecological Applications,* 13(2), 461-473.

Jackson, J.K. and Fuereder, L. (2006) Long-term studies of freshwater macroinvertebrates: a review of the frequency, duration and ecological significance. *Freshwater biology*, *51*(3), 591-603.

Jacobsen, D. and Encalada, A. (1998) The macroinvertebrate fauna of Ecuadorian highland streams in the wet and dry season. *Archiv Fur Hydrobiologie,* 142(1), 53-70.

Jacobsen, D., C. Cressa, J. M. Mathooko, and D. Dudgeon. 2008. Macroinvertebrates: Composition, Life Histories and Production. Pages 65-105 *in* D. Dudgeon, editor. Tropical Stream Ecology. Academic Press, London.

James, F. C. and McCulloch, C. E. (1990) Multivariate analysis in ecology and systematics: panacea or Pandora's box? *Annual Review of Ecology and Systematics,* 21, 129-166.

Jinggut, T., 2015. *Leaf litter breakdown in streams of East Malaysia, Borneo*: *a study of biodiversity and ecosystem functioning.* Monash University Sunway Campus.

Jönck, C. R. and Aranha, J. M. R. (2010) Influence of a waterfall over richness and similarity in adjoining pools of an Atlantic Rainforest stream. *Acta Limnologica Brasiliensia,* 22(4), 378-383.

Jowett, I. G. (1993) A method for objectively identifying pool, run, and riffle habitats from physical measurements. New Zealand journal of marine and freshwater research 27: 241-248.

Junk, W. J., Bayley, P. B. and Sparks, R. E. (1989) The flood pulse concept in river-floodplain systems. *Canadian special publication of fisheries and aquatic sciences,* 106(1), 110-127.

Kano, Y., Nishida, S. and Nakajima, J. (2012) Waterfalls drive parallel evolution in a freshwater goby. *Ecology and Evolution,* 2(8), 1805-1817.

Karssing, R. J., Rivers-Moore N. A. and Slater K. (2012) Influence of waterfalls on patterns of association between trout and Natal cascade frog Hadromophryne natalensis tadpoles in two headwater streams in the uKhahlamba Drakensberg Park World Heritage Site, South Africa. *African Journal of Aquatic Science* 37:107-112.

Kiffney, P. M., Greene, C. M., Hall, J. E. and Davies, J. R. (2006) Tributary streams create spatial discontinuities in habitat, biological productivity, and diversity in mainstem rivers. *Canadian Journal of Fisheries and Aquatic Sciences,* 63(11), 2518-2530.

Knispel, S. and Castella, E. (2003) Disruption of a longitudinal pattern in environmental factors and benthic fauna by a glacial tributary. *Freshwater Biology,* 48(4), 604-618.

Kottelat, M., (2013) The Fishes of the Inland Waters of Southeast Asia: A Catalogue and Core Bibliography of the Fishes Known to Occur in Freshwaters, Mangroves and Estuaries. *Raffles Bulletin of Zoology*. 27, 1-663.

Kovalenko, K., Thomaz, S. and Warfe, D. (2012) Habitat complexity: approaches and future directions. *Hydrobiologia,* 685(1), 1-17.

Kundzewicz, Z. W. (2002) Ecohydrology-seeking consensus on interpretation of the notion. *Hydrological Sciences-Journal-des Sciences Hydrologiques*, 47(5), 799-804.

Labonne, J. and Hendry, A. P. (2010) Natural and Sexual Selection Giveth and Taketh Away Reproductive Barriers: Models of Population Divergence in Guppies. *The American Naturalist,* 176(1), 26-39.

Lafferty, K. D. and Kuris, A. M.. (2002) Trophic strategies, animal diversity and body size. *Trends in Ecology & Evolution*, *17*(11), 507-513.

Lake, P. S. (1990) Disturbing hard and soft bottom communities: a comparison of marine and freshwater environments. *Australian Journal of Ecology,* 15(4), 477-488.

Lake, P. S., Bond, N. and Reich, P. (2007) Linking ecological theory with stream restoration. *Freshwater Biology,* 52(4), 597-615.

Lake, P. S., Schreiber, E. S. G., Milne, B. J. and Pearson, R. G. (1994) Species richness in streams: patterns over time, with stream size and with latitude. *Internationale Vereinigung fur Theoretische und Angewandte Limnologie Verhandlungen,* 25(3), 1822-1826.

Lamberti, G. A. and Resh, V. H. (1983) Stream Periphyton and Insect Herbivores: An Experimental Study of Grazing by a Caddisfly Population. *Ecology,* 64(5), 1124-1135.

Lamouroux, N., Mérigoux, S., Capra, H., Dolédec, S., Jowett, I. G. and Statzner, B. (2010) The generality of abundance-environment relationships in microhabitats: A comment on Lancaster and Downes (2009). *River Research and Applications,* 26(7), 915-920.

Lancaster, J. and Downes, B. J. (2010) Linking the hydraulic world of individual organisms to ecological processes: Putting ecology into ecohydraulics. *River Research and Applications,* 26(4), 385-403.

Landeiro, V. L., Hamada N. and Melo A. S. (2008) Responses of aquatic invertebrate assemblages and leaf breakdown to macroconsumer exclusion in Amazonian "terra firme" streams. *Fundamental and Applied Limnology* 172:49-58.

Lau, D. C. P., Leung, K. M. Y. and Dudgeon, D. (2009a) Are autochthonous foods more important than allochthonous resources to benthic consumers in tropical headwater streams? *Journal of the North American Benthological Society,* 28(2), 426-439.

Lau, D. C. P., Leung, K. M. Y. and Dudgeon, D. (2009b) What does stable isotope analysis reveal about trophic relationships and the relative importance of allochthonous and autochthonous resources in tropical streams? A synthetic study from Hong Kong. *Freshwater Biology,* 54(1), 127-141.

Layer, K., Hildrew, A. G. and Woodward, G. (2012) Grazing and detritivory in 20 stream food webs across a broad pH gradient. *Oecologia,* 171(2), 459-471.

Lecerf, A. and Richardson, J. (2009) Biodiversity-ecosystem function research: Insights gained from streams. *River research and applications,* 26(1), 45-54.

Leclerc, M. (2002). *Ecohydraulics, last frontier for fluvial hydraulics: research challenges and multidisciplinary perspectives*. Paper presented at the Proceedings of RiverFlow 2002 Conference, Louvain-la-Neuve, Belgium.

Leung, A. S. L., and D. Dudgeon. (2011) Scales of spatiotemporal variability in macroinvertebrate abundance and diversity in monsoonal streams: Detecting environmental change. Freshwater Biology. 56:1193–1208.

Lewis S. L., D. P. Edwards, and D. Galbraith (2015) Increasing human dominance of tropical forests. *Science* 349:827-832.

Lewis, J., W.M. (2008) Physical and Chemical Features of Tropical Flowing Waters. *In:* D. Dudgeon (Ed) *Tropical Stream Ecology*. London: Academic Press.

Li, A. O. Y. and Dudgeon, D. (2008) Food resources of shredders and other benthic macroinvertebrates in relation to shading conditions in tropical Hong Kong streams. *Freshwater Biology,* 53(10), 2011-2025.

Livingston, B. E. (1903) The Distribution of the Upland Plant Societies of Kent County, Michigan." *Botanical Gazette* 35: 36–55. From: Amoros, C., Gibert, J. and Greenwood, M. (1996) Interactions between units of the fluvial hydrosystem *The Fluvial Hydrosystems*. Springer, 1996, 184-210.

Logan, P. and Furse, M. (2002) Preparing for the European Water Framework Directive - making the links between habitat and aquatic biota. *Aquatic Conservation: Marine and Freshwater Ecosystems,* 12(4), 425-437.

Lucas, M. C., & Baras E. (2001) *Migration of freshwater fishes*, Blackwell Science Ltd, Oxford.

Mac Nally, R., Wallis, E. and Lake, P. S. (2011) Geometry of biodiversity patterning: assemblages of benthic macroinvertebrates at tributary confluences. *Aquatic Ecology,* 45(1), 43-54.

Mackay, R. J. (1992) Colonization by Lotic Macroinvertebrates: A Review of Processes and Patterns. *Canadian Journal of Fisheries and Aquatic Sciences,* 49(3), 617-628.

Mantel, S. K., Salas, M. and Dudgeon, D. (2004) Foodweb structure in a tropical Asian forest stream. *Journal of the North American Benthological Society,* 23(4), 728-755.

March, J. G. and Pringle, C. M. (2003) Food Web Structure and Basal Resource Utilization along a Tropical Island Stream Continuum, Puerto Rico. *Biotropica,* 35(1), 84-93.

March, J. G., Benstead, J. P., Pringle, C. M. and Ruebel, M. W. (2001) Linking shrimp assemblages with rates of detrital processing along an elevational gradient in a tropical stream. *Canadian journal of fisheries and aquatic sciences,* 58(3), 470-478.

March, J. G., Pringle, C. M., Townsend, M. J. and Wilson, A. I. (2002) Effects of freshwater shrimp assemblages on benthic communities along an altitudinal gradient of a tropical island stream. *Freshwater Biology,* 47(3), 377-390.

Marchant, R., Lake, P. S. and Doeg, T. J. (1991) Longitudinal variation in recolonization rates of macroinvertebrates along an upland river in south-eastern Australia. *Freshwater Biology,* 25(2), 349-356.

Masese, F. O., Kitaka, N. Kipkemboi, J. Gettel, G. M Irvine K. and McClain M. E. (2014) Macroinvertebrate functional feeding groups in Kenyan highland streams: evidence for a diverse shredder guild. *Freshwater Science* 33:435-450.

Mathooko, J. M. and Kariuki, S. T. (2000) Disturbances and species distribution of the riparian vegetation of a Rift Valley stream. *African Journal of Ecology* 38:123-129.

Mathooko, J. M., (1998) The effect of continuous physical disturbance on mayflies of a tropical stream: an experimental approach. *Hydrobiologia* 362:209-216.

Mazlan, E. H. B. A. F. and Kamarudin, S. J. (2015) Diversity, Composition and Distribution of Aquatic Insects in Liwagu Water Catchment, Tambunan, Sabah. *Journal of Tropical Biology and Conservation,* 12, 13-24.

McConnell, R. and Lowe-McConnell R.H., (1987) *Ecological studies in tropical fish communities.* Cambridge University Press, Cambridge.

McGuire, T. L. and Winemiller, K. O. (1998) Occurrence Patterns, Habitat Associations, and Potential Prey of the River Dolphin, Inia geoffrensis, in the Cinaruco River, Venezuela1. *Biotropica,* 30(4), 625-638.

McKie, B. G. (2004) Disturbance and investment: developmental responses of tropical lotic midges to repeated tube destruction in the juvenile stages. *Ecological Entomology,* 29(4), 457-466.

McManamay, R. A., Bevelhimer M. S., and Kao S. C. (2014) Updating the US hydrologic Classification: An Approach to Clustering and Stratifying Ecohydrologic Data. *Ecohydrology* 7:903-926.

McNeely, C., Finlay, J. C. and Power, M. E. (2007) Grazer traits, competition, and carbon sources to a headwater-stream food web. *Ecology,* 88(2), 391-401.

Md Rawi, C. S., Al-Shami, S. A., Madrus, M. R., and Ahmad, A. H. (2014) Biological and ecological diversity of aquatic macroinvertebrates in response to hydrological and

physicochemical parameters in tropical forest streams of Gunung Tebu, Malaysia: implications for ecohydrological assessment. *Ecohydrology* 7:496-507.

Melles, S. J., Jones, N. E. and Schmidt, B. (2012) Review of theoretical developments in stream ecology and their influence on stream classification and conservation planning. *Freshwater Biology,* 57(3), 415-434.

Menezes, S., Baird, D. J. and Soares, A. M. V. M. (2010) Beyond taxonomy: a review of macroinvertebrate trait-based community descriptors as tools for freshwater biomonitoring. *Journal of Applied Ecology,* 47(4), 711-719.

Miettinen, J., Shi, C. and Liew S. C. (2011) Deforestation rates in insular Southeast Asia between 2000 and 2010. *Global Change Biology* 17: 2261-2270.

Miettinen, J., Shi, C. and Liew, S. C. (2011) Two decades of destruction in Southeast Asia's peat swamp forests. *Frontiers in Ecology and the Environment,* 10(3), 124-128.

Minshall, G. W., Cummins, K. W., Petersen, R. C., Cushing, C. E., Bruns, D. A., Sedell, J. R. and Vannote, R. L. (1985) Developments in stream ecosystem theory. *Canadian Journal of Fisheries and Aquatic Sciences,* 42(5), 1045-1055.

Mittermeier, R. A., Myers, N., Gil, P. R. and Mittermeier, C. G. (1999) *Hotspots: Earth's Biologically Richest and Most Endangered Terrestrail Ecoregions*. Chicago: University of Chicago Press.

Montgomery, D. R. (1999) Process domains and the river continuum *Journal of the American Water Resources Association,* 35(2), 397-410.

Moulton, T. P., Souza, M. L., Silveira, R. M. L., Krsulović, F. A. M., Silveira, M. P., de Assis, J. C. F. and Francischetti, C. N. (2010) Patterns of periphyton are determined by cascading trophic relationships in two neotropical streams. *Marine and Freshwater Research,* 61(1), 57-64.

Moulton, T.P., Lourenço-Amorim, C., Sasada-Sato, C.Y., Neres-Lima, V. and Zandonà, E., (2015) Dynamics of algal production and ephemeropteran grazing of periphyton in a tropical stream. *International Review of Hydrobiology*, *100*(2), 61-68.

Neill, C., Deegan, L. A., Thomas, S. M. and Cerri, C. C. (2001) Deforestation for pasture alters nitrogen and phosphorus in small Amazonian streams. *Ecological Applications,* 11(6), 1817-1828.

Nellemann, C. (2007) *The last stand of the orangutan: state of emergency: Illegal logging, fire and palm oil in Indonesia's National Parks*. UNEP/Earthprint.

Newson, M. (2002) Geomorphological concepts and tools for sustainable river ecosystem management. *Aquatic Conservation: Marine and Freshwater Ecosystems,* 12(4), 365-379.

Newson, M. and Newson, C. (2000) Geomorphology, ecology and river channel habitat: mesoscale approaches to basin-scale challenges. *Progress in Physical Geography,* 24(2), 195-217.

Nielsen, A. (1950) The Torrential Invertebrate Fauna. *Oikos,* 2(2), 176-196.

Odland, A., Birks, H. H., Botnen, A., Tønsberg, T. and Vevle, O. (1991) Vegetation change in the spray zone of a waterfall following river regulation in Aurland, western Norway. *Regulated Rivers: Research & Management,* 6(3), 147-162.

O'Keeffe, J. H., King, J. and Eekhout, S. (1994). *The characteristics and purposes of river classification*. Paper presented at the Proceedings of a Joint South African/Australian Workshop, February 7-14 1994, Cape Town, South Africa. WRC Report No. TT 63/94.

Oksanen, J. (2016) Vegan: an introduction to ordination. http://cran. r-project. org/web/packages/vegan/vignettes/introvegan. pdf.

Olson, A. D. and West, J. (1989) *Evaluation of Instream Fish Habitat Restoration Structures in Klamath River Tributaries, 1988/1989*. US Department of Agriculture, Forest Service, Pacific Southwest Region.

Ormerod, S. J., Rundle, S. D., Wilkinson, S. M., Daly, G. P., Dale, K. M. and Juttner, I. (1994) Altitudinal trends in the diatoms, bryophytes, macroinvertebrates and fish of a Nepalese river system. *Freshwater Biology,* 32(2), 309-322.

Orr, A. G. (2001) An annotated checklist of the Odonata of Brunei with ecological notes and descriptions of hitherto unknown males and larvae. *International Journal of Odonatology,* 4(2), 167-220.

Ortiz-Zayas, J. R., Lewis Jr, W. M., Saunders III, J. F., McCutchan Jr, J. H. and Scatena, F. N. (2005) Metabolism of a tropical rainforest stream. *Journal of the North American Benthological Society,* 24(4), 769-783.

Osborne, L. L. and Wiley, M. J. (1992) Influence of Tributary Spatial Position on the Structure of Warm water Fish Communities. *Canadian Journal of Fisheries and Aquatic Sciences,* 49(4), 671-681.

Osterkamp, W. (2002) Geoindicators for river and river-valley monitoring in the humid tropics. *Environmental Geology,* 42(7), 725-735.

Padmore, C. (1998) The role of physical biotopes in determining the conservation status and flow requirements of British rivers. *Aquatic Ecosystem Health & Management,* 1(1), 25-35.

Palmer, M. A., Ambrose, R. F. and Poff, N. L. (1997) Ecological Theory and Community Restoration Ecology. *Restoration Ecology,* 5(4), 291-300.

Parasiewicz, P. (2007) The MesoHABSIM model revisited. River Research and Applications 23:893-903.

Pearson, R. G. (2014) Dynamics of invertebrate diversity in a tropical stream. *Diversity* 6: 771-791.

Pearson, R. G., Benson, L. J. and Smith, R. E. W. (1986) Diversity and Abundance of the Fauna in Yuccabine Creek, a Tropical Rainforest Stream. *In:* P. Deckker & W. D. Williams Eds. *Limnology in Australia*. Vol. 61: Springer Netherlands, 1986, 329-342.

Pearson, R., Tobin, R., Smith, R. and Benson, L. (1989) Standing crop and processing of rainforest litter in a tropical Australian stream. *Archiv für Hydrobiologie,* 115(4), 481-498.

Peckarsky, B. L., Horn, S. C. and Statzner, B. (1990) Stonefly predation along a hydraulic gradient: a field test of the harsh—benign hypothesis. *Freshwater Biology,* 24(1), 181-191.

Pendry, C. A. and Proctor, J. (1996) The Causes of Altitudinal Zonation of Rain Forests on Bukit Belalong, Brunei. *Journal of Ecology,* 84(3), 407-418.

Petersen, R. C. and Cummins, K. W. (1974) Leaf processing in a woodland stream. *Freshwater biology,* 4(4), 343-368.

Peterson, B. J. and Fry, B. (1987) Stable isotopes in ecosystem studies. *Annual review of ecology and systematics*, 293-320.

Petts, G. E. and Amoros, C. (1996) *The fluvial hydrosystem*. Springer, Netherlands.

Petts, G. E., Maddock, I., Bickerton, M. and Ferguson, A. J. D. (1995) Linking hydrology and ecology: the scientific basis for river management. *In:* D. Harper & A. J. D. Ferguson Eds. *The Ecological Basis for River Management*. Chichester: John Wiley & Sons, 1995, 1-16.

Pickett, S. T. A. and White, P. S. (1985) *The ecology of natural disturbance and patch dynamics*. Academic Press: Orlando, Florida.

Poff, N. L. and Ward, J. V. (1989) Implications of streamflow variability and predictability for lotic community structure: a regional analysis of streamflow patterns. *Canadian journal of fisheries and aquatic sciences*, *46*(10), 1805-1818.

Poff, N. L. R. (1997) Landscape filters and species traits: towards mechanistic understanding and prediction in stream ecology. *Journal of the north american Benthological society*, 391-409.

Poff, N. L. R. and Ward, J. (1995) Herbivory under different flow regimes: a field experiment and test of a model with a benthic stream insect. *Oikos*, 179-188.

Poff, N. L. R., Olden, J. D., Vieira, N. K. M., Finn, D. S., Simmons, M. P. and Kondratieff, B. C. (2006) Functional trait niches of North American lotic insects: traits-based ecological applications in light of phylogenetic relationships. *Journal of the North American Benthological Society,* 25(4), 730-755.

Polgar, G., Khang, T. F., Chua, T. and Marshall, D. J. (2015) Gross mismatch between thermal tolerances and environmental temperatures in a tropical freshwater snail: Climate warming and evolutionary implications. *Journal of Thermal Biology,* 47, 99-108.

Poole, G. C. (2002) Fluvial landscape ecology: addressing uniqueness within the river discontinuum. *Freshwater Biology,* 47(4), 641-660.

Poole, G. C. (2010) Stream hydrogeomorphology as a physical science basis for advances in stream ecology. *Journal of the North American Benthological Society* 29:12-25.

Power, M. E. (1984) The importance of sediment in the grazing ecology and size class interactions of an armored catfish, Ancistrus spinosus. *Environmental Biology of Fishes,* 10(3), 173-181.

Power, M. E. and Dietrich, W. E. (2002) Food webs in river networks. *Ecological Research,* 17(4), 451-471.

Power, M. E., Stout, R. J., Cushing, C. E., Harper, P. P., Hauer, F. R., Matthews, W. J., Moyle, P. B., Statzner, B., Irene, R. and De Badgen, W. (1988) Biotic and abiotic controls in river and stream communities. *Journal of the North American Benthological Society*, 1:456-479.

Power, M. E., Stout, R. J., Cushing, C. E., Harper, P. P., Hauer, F. R., Matthews, W. J., Moyle, P. B., Statzner, B. and Badgen, I. R. W. D. (1988) Biotic and Abiotic Controls in River and Stream Communities. *Journal of the North American Benthological Society,* 7(4), 456-479.

Price, P. W. (1975) *Insect Ecology*. Wiley, New York.

Principe R. E. (2008) Taxonomic and size structures of aquatic macroinvertebrate assemblages in different habitats of tropical streams, Costa Rica. *Zoologica Studies* 47:525-534.

Pringle, C. M. (1996) Atyid shrimps (Decapoda: Atyidae) influence the spatial heterogeneity of algal communities over different scales in tropical montane streams, Puerto Rico. *Freshwater Biology,* 35(1), 125-140.

Pringle, C. M. (1998) Structure and Production of a Benthic Insect Assemblage in a Neotropical Stream. *Journal of the North American Benthological Society* 17: 443-463.

Pringle, C. M. and Hamazaki, T. (1997) Effects of fishes on algal response to storms in a tropical stream. *Ecology,* 78(8), 2432-2442.

Pringle, C. M. and Hamazaki, T. (1998) The role of omnivory in a neotropical stream: separating diurnal and nocturnal effects. *Ecology,* 79(1), 269-280.

Pringle, C. M., Naiman, R. J., Bretschko, G., Karr, J. R., Oswood, M. W., Webster, J. R., Welcomme, R. L. and Winterbourn, M. J. (1988) Patch dynamics in lotic systems: the stream as a mosaic. *Journal of the North American Benthological Society*, 503-524.

Pringle, C.M., Blake, G.A., Covich, A.P., Buzby, K.M. and Finley, A. (1993) Effects of omnivorous shrimp in a montane tropical stream: sediment removal, disturbance of sessile invertebrates and enhancement of understory algal biomass. *Oecologia*, *93*(1): 1-11.

Quentin DS. (1973) Results of the Austrian-Ceylonese Hydrobiological Mission 1970. Part XII. Contributions to the ecology of the larvae of some Odonata from Ceylon. *Bull Fish Res Stn Sri Lanka (Ceylon),* 24:113-124.

Rabeni, C. F. and Jacobson, R. B. (1993) The importance of fluvial hydraulics to fish-habitat restoration in low-gradient alluvial streams. *Freshwater Biology,* 29(2), 211-220.

Rabeni, C. F. and Minshall, G. W. (1977) Factors affecting microdistribution of stream benthic insects. *Oikos*, 29: 33-43.

Rackemann, S. L., Robson, B. J. and Matthews, T. G. (2013) Conservation value of waterfalls as habitat for lotic insects of western Victoria, Australia. *Aquatic Conservation: Marine and Freshwater Ecosystems,* 23(1), 171-178.

Ramírez A, and Pringle C. M. (1998) Structure and production of a benthic insect assemblage in a neotropical stream. *Journal North American Benthological Society* 17: 443–463

Ramirez, A. and Hernandez-Cruz, L. R. (2004) Aquatic Insect Assemblages in Shrimp-dominated Tropical Streams, Puerto Rico. *Biotropica,* 36(2), 259-266.

Ramírez, A. and Pringle, C. M. (1998) Structure and Production of a Benthic Insect Assemblage in a Neotropical Stream. *Journal of the North American Benthological Society,* 17(4), 443-463.

Ramírez, A., M. Ardón, M. M. Douglas, and M. A. S Graça. (2015) Tropical freshwater sciences: an overview of ongoing tropical research. *Freshwater Science* 34:606-608.

Ramirez, A., Paaby, P., Pringle, C. M. and Agüero, G. (1998) Effect of habitat type on benthic macroinvertebrates in two lowland tropical streams, Costa Rica. *Revista de biología tropical,* 46, 201-214.

Ramirez, A., Pringle, C. M., Wantzen, K. M. and Dudgeon, D. (2008) Tropical stream conservation *In:* D. Dudgeon Ed. *Tropical Stream Ecology*. Academic Press, London.

Resh, V. H., Brown, A. V., Covich, A. P., Gurtz, M. E., Li, H. W., Minshall, G. W., Reice, S. R., Sheldon, A. L., Wallace, J. B. and Wissmar, R. C. (1988) The Role of Disturbance in Stream Ecology. *Journal of the North American Benthological Society,* 7(4), 433-455.

Resh, V. H., Hildrew, A. G., Statzner, B. and Townsend, C. (1994) Theoretical habitat templets, species traits, and species richness - a synthesis of long-term ecological research on the Upper Rhone River in the context of concurrently developed ecological theory. *Freshwater Biology* 31, 539-554.

Rice, P., Lancaster, J. and Kemp, P. (2010) Experimentation at the interface of fluvial geomorphology, stream ecology and hydraulic engineering and the development of an effective, interdisciplinary river science. *Earth Surface Processes and Landforms,* 35(1), 64-77.

Rice, S. P., Greenwood, M. T. and Joyce, C. B. (2001) Tributaries, sediment sources, and the longitudinal organisation of macroinvertebrate fauna along river systems. *Canadian Journal of Fisheries and Aquatic Sciences,* 58(4), 824-840.

Rice, S. P., Kiffney, P., Greene, C. and Pess, G. R. (2008a) The ecological importance of tributaries and confluences. In Rice, S. P., Roy, A. G. and Rhoads, B. L. (2008) *River confluences, tributaries and the Fluvial Network*. Wiley Online Library.

Rice, S. P., Rhoads, B. L. and Roy, A. G. (2008b) Introduction: river confluences, tributaries and the fluvial network In: Rice, S. P., Roy, A. G. and Rhoads, B. L. (2008) *River confluences, tributaries and the Fluvial Network*. Wiley Online Library.

Riley, S. C. and Fausch, K. D. (1995) Trout population response to habitat enhancement in six northern Colorado streams. *Canadian Journal of Fisheries and Aquatic Sciences,* 52(1), 34-53.

Rodríguez-Iturbe, I. and Rinaldo, A. (2001) *Fractal river basins: chance and self-organization*. Cambridge University Press.

Roni, P. and Quinn, T. P. (2001) Density and size of juvenile salmonids in response to placement of large woody debris in western Oregon and Washington streams. *Canadian Journal of Fisheries and Aquatic Sciences,* 58(2), 282-292.

Rosales, J., Blanco-Belmonte, L., Bradley, C. (2008) Hydrogeomorphological and ecological interactions in tropical floodplains: the significance of confluence zones in the Orinoco basin, Venezuela. In: Wood, P.J., Hannah, D.M., Sadler, J.P. (Eds.), *Hydroecology and Ecohydrology: Past, Present and Future*. J. Wiley, Chichester, 295-316.

Rosemond, A. D., Pringle, C. M. and Ramírez, A. (1998) Macroconsumer effects on insect detritivores and detritus processing in a tropical stream. *Freshwater Biology,* 39(3), 515-523.

Rosi-Marshall, E. J. and Wallace, J. B. (2002) Invertebrate food webs along a stream resource gradient. *Freshwater Biology,* 47(1), 129-141.

Ross, S. T. (2013) *Ecology of North American Freshwater Fishes*. London: University of California Press.

Rosser, Z. C. and Pearson, R. G. (1995) Responses of Rock Fauna to Physical Disturbance in Two Australian Tropical Rainforest Streams. *Journal of the North American Benthological Society,* 14(1), 183-196.

Rueda-Delgado, G., Wantzen, K. M. and Tolosa, M. B., (2006) Leaf-litter decomposition in an Amazonian floodplain stream: effects of seasonal hydrological changes. *Journal of the North American Benthological Society*, 25(1), 233-249.

Rundle, S. D., Jenkins, A. and Ormerod, S. J. (1993) Macroinvertebrate communities in streams in the Himalaya, Nepal. *Freshwater Biology,* 30(1), 169-180.

Sabo, J. L. and Power, M. E. (2002) Numerical response of lizards to aquatic insects and short-term consequences for terrestrial prey. *Ecology,* 83(11), 3023-3036.

Sabo, J. L., Bastow, J. L. and Power, M. E. (2002) Length–mass relationships for adult aquatic and terrestrial invertebrates in a California watershed. *Journal of the North American Benthological Society,* 21(2), 336-343.

Sangpradub, N., & Boonsoong, B. (2006). *Identification of freshwater invertebrates of the Mekong river and tributaries*. Mekong River Commission, Vientiane.

Schmid P. E., Tokeshi, M, and Schmid-Araya, J. M. (2000) Relation between population density and body size in stream communities. *Science* 289:1557-1560.

Schoener T. W. (1986) Overview: kinds of ecological communities-ecology becomes pluralistic. In: Diamond J. Case T. J (ed) *Community ecology*. Harper and Row, New York.

Schuwirth, N. and Reichert, P. (2012) Predicting the occurence of macroinvertebrates. *Eawag News*(72), 14-17.

Sehal, K. L. (1983) Fishery resources and their management *In:* T. V. Singh & J. kaur Eds. *Studies in Ecodevelopment: Himalayas Mountains and Men*. Lucknow: Print House, 1983, 225-272.

Serra-Tosio, B., (1967) La prise de nourriture chez la larve de Prosimulium inflatum Davies, 1957 (Diptera, Simuliidae). *Traveaux du Laboratoire d'hydrobiologie et de pisciculture de l'Universitié de Grenoble*, pp.57-58. From: Wotton, R. S. (1977) The Size of Particles Ingested by Moorland Stream Blackfly Larvae (Simuliidae). *Oikos,* 29(2), 332-335.

Sheldon, A. L. (2011) Comparative habitat use by grazing fishes in a Bornean stream. *Environmental Biology of Fishes,* 92(3), 381-390.

Sivaramakrishnan, K. G. and Venkataraman, K. (1990) Abundance, altitudinal distribution and swarming of Ephemeroptera in Palini Hills, South India. *In:* I. C. Campbell Ed. *Mayflies and Stoneflies: Life Histories and Biology*. Dordrecht: Kluwer Academic Publishers, 1990.

Smock, L. A., J. E. Gladden, J. L. Riekenberg, L. C. Smith, and C. R Black. (1992) Lotic Macroinvertebrate Production in Three Dimensions: Channel Surface, Hyporheic, and Floodplain Environments. Ecology 73: 876-886.

Sodhi, N. S., Koh, L. P., Brook, B. W. and Ng, P. K. L. (2004) Southeast Asian biodiversity: an impending disaster. *Trends in Ecology & Evolution,* 19(12), 654-660.

St Quentin, D. S. (1973) Results of the Austrian-Ceylonese hydrobiological mission, 1970. Part XII: Contributions to the Ecology of the Larvae of some Odonata from Ceylon. *Bulletin of the Fisheries Research Station, Sri Lanka,* 24, 113-124.

Stanford, J. A. and Ward, J. (1993) An ecosystem perspective of alluvial rivers: connectivity and the hyporheic corridor. *Journal of the North American Benthological Society*, 48-60.

Stark, B. P., & Sheldon, A. L. (2009) Records of Neoperlini (Plecoptera: Perlidae) from Brunei Darussalam and Sarawak, with descriptions of new Phanoperla Banks and Neoperla Needham species. *Illiesia*, *5*, 11-19.

Statzner, B. and Bêche, L. A. (2010) Can biological invertebrate traits resolve effects of multiple stressors on running water ecosystems? *Freshwater Biology,* 55, 80-119.

Statzner, B. and Higler, B. (1986) Stream hydraulics as a major determinant of benthic invertebrate zonation patterns. *Freshwater Biology,* 16(1), 127-139.

Statzner, B., Resh, V. H. and Dolédec, S. (1994) *Ecology of the Upper Rhône River: a test of habitat templet theories*. Blackwell Scientific.

Steinman, A. D, S. (1996) Effects of grazers on freshwater benthic algae. In: Stevenson, R., M. I. Bothwell and R. L. Lowe (Eds). *Algal ecology: freshwater benthic ecosystems* Academic Press, San Diego 341–366.

Steinmann, P. (1907) Die Tierwelt der Gebirgsbäche. Eine faunistischbiologische Studie. *Annales de Biologie Lacustre,* 2, 30–150.

Steinmann, P. (1908) Die Tierwelt der Gebirgsbäche. *Archiv für Hydrobiologie* 35, 266–273

Stevens, L. E., Shannon, J. P. and Blinn, D. W. (1997) Colorado River benthic ecology in Grand Canyon, Arizona, USA: dam, tributary and geomorphological influences. *Regulated rivers: research & management,* 13(2), 129-149.

Stout, J. and Vandermeer, J. (1975) Comparison of species richness for stream-inhabiting insects in tropical and mid-latitude streams. *American Naturalist*, 263-280.

Stubbington, R, Greenwood A. M, Wood P. J, Armitage P. D, Gunn, J, Robertson A. L. (2009) The response of perennial and temporary headwater stream invertebrate communities to hydrological extremes. *Hydrobiologia* 630:299-312.

Tews, J., U. Brose, V. Grimm, K. Tielbörger, M. C. Wichmann, M. Schwager and F. Jeltsch (2004). Animal species diversity driven by habitat heterogeneity/diversity: the importance of keystone structures. *Journal of Biogeography* 31(1): 79-92.

Thomas, R., Vaughan, I. and Lello, J. (2013) *Data analysis with R statistical software: a guidebook for scientists*. Eco-explore.

Todorova, Y. and Topalova, Y. (2014) Importance of Main Stream/Tributary Interface for Nutrient Dynamics and Microbial Communities in Upper River Subcatchment. *Annual Research & Review in Biology,* 4(2), 399.

Tonnoir, A. (1930) Notes on Indian Blepharocerid larvae and pupae with remarks on the morphology of Blepharocerid larvae and pupae in general. *Records of the Indian Museum,* 32, 161-214.

Torrente-Vilara, G., Zuanon, J., Leprieur, F., Oberdorff, T. and Tedesco, P. A. (2011) Effects of natural rapids and waterfalls on fish assemblage structure in the Madeira River (Amazon Basin). *Ecology of Freshwater Fish,* 20(4), 588-597.

Townsend, C. (1996) Concepts in river ecology: pattern and process in the catchment hierarchy. *Archiv für Hydrobiologie. Supplementband. Large rivers,* 10(1-4), 3-21.

Townsend, C. and Hildrew, A. G. (1994) Species traits in relation to a habitat templet for river systems. *Freshwater Biology,* 31(3), 265-275.

Udvardy, M. F. (1959) Notes on the ecological concepts of habitat, biotope and niche. *Ecology*, 725-728.

Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R. and Cushing, C. E. (1980) The river continuum concept. *Canadian journal of fisheries and aquatic sciences,* 37(1), 130-137.

Vaughan, I. P., Diamond, M., Gurnell, A., Hall, K., Jenkins, A., Milner, N., Naylor, L., Sear, D., Woodward, G. and Ormerod, S. (2009) Integrating ecology with hydromorphology: a priority for river science and management. *Aquatic Conservation: Marine and Freshwater Ecosystems,* 19(1), 113-125.

Verberk, W. C. E. P., van Noordwijk, C. G. E. and Hildrew, A. G. (2013) Delivering on a promise: integrating species traits to transform descriptive community ecology into a predictive science. *Freshwater Science,* 32(2), 531-547.

Villeneuve, B., Souchon Y., Usseglio-Polatera P., Ferréol, M. and Valette L. (2015) Can we predict biological condition of stream ecosystems? A multi-stressors approach linking three biological indices to physico-chemistry, hydromorphology and land use. *Ecological Indicators* 48:88-98.

Vinson, M. R. and Hawkins, C. P. (1998) Biodiversity of Stream Insects: Variation at Local, Basin, and Regional Scales. *Annual Review of Entomology,* 43(1), 271-293.

Wadeson RA. (1995) The Development of the Hydraulic Biotope Concept within a Catchment Based Hierarchical Geomorphological Model. PhD Dissertation. Rhodes University.

Wallace, J. B., Cuffney, T., Webster, J., Lugthart, G., Chung, K. and Goldowitz, B. (1991) Export of fine organic particles from headwater streams: effects of season, extreme discharges, and invertebrate manipulation. *Limnology and Oceanography,* 36(4), 670-682.

Wallace, J. B., Eggert, S. L., Meyer, J. L. and Webster, J. R. (1997) Multiple trophic levels of a forest stream linked to terrestrial litter inputs. *Science,* 277(5322), 102-104.

Wallis, E., Nally, R. M. and Lake, P. S. (2008) A Bayesian analysis of physical habitat changes at tributary confluences in cobble-bed upland streams of the Acheron River basin, Australia. *Water Resources Research,* 44(11).

Wantzen, K. M., Yule, C. M., Mathooko, J. M. and Pringle, C. M. (2008) Organic Matter Processing in Tropical Streams. In: D. Dudgeon Ed. *Tropical Stream Ecology*. London: Academic Press, 2008.

Ward, J. (1989) The four-dimensional nature of lotic ecosystems. *Journal of the North American Benthological Society*, 2-8.

Ward, J. V. (1992) *Aquatic insect ecology: 1. biology and habitat*. New York: John Wiley & Sons.

Ward, J. V. and Stanford, J. (1983) The serial discontinuity concept of lotic ecosystems. *Dynamics of lotic ecosystems*, 29-42.

Ward, J. V. and Stanford, J. A. (1995) Ecological connectivity in alluvial river ecosystems and its disruption by flow regulation. *Regulated Rivers: Research & Management,* 11(1), 105-119.

Ward, J. V. and Weins, J. A. (2001) Ecotones of river ecosystems: Role and typology, spatio-temporal dynamics and river regulation. *Ecohydrology and Hydrobiology,* 1, 25-36.

Waters, T. F. (1977) Secondary production in inland waters. *Advances in ecological research,* 10, 91-164.

Wellnitz, T. and Leroy Poff, N. (2006) Herbivory, current velocity and algal regrowth: how does periphyton grow when the grazers have gone? *Freshwater Biology,* 51(11), 2114-2123.

Wetzel, R. G. (2001) Limnology: lake and river ecosystems, 3rd edn. San Diego:

Whiles, M. R., Hall, R. O., Jr., Dodds, W. K., Verburg, P., Huryn, A. D., Pringle, C. M., Lips, K. R., Kilham, S. S., Colón-Gaud, C., Rugenski, A. T., Peterson, S. and Connelly, S. (2013) Disease-Driven Amphibian Declines Alter Ecosystem Processes in a Tropical Stream. *Ecosystems,* 16(1), 146-157.

White, A. F., Blum, A. E., Schulz, M. S., Vivit, D. V., Stonestrom, D. A., Larsen, M., Murphy, S. F. and Eberl, D. (1998) Chemical weathering in a tropical watershed, Luquillo Mountains, Puerto Rico: I. Long-term versus short-term weathering fluxes. *Geochimica et Cosmochimica Acta,* 62(2), 209-226.

White, E.P., Ernest, S.M., Kerkhoff, A.J. and Enquist, B.J., (2007) Relationships between body size and abundance in ecology. *Trends in ecology & evolution*, *22*(6): 323-330.

White, M. and Klum, M. (2008) Borneo's moment of truth. *National Geographic,* 214(5), 37-63.

Whittaker, R. H., Levin, S. A. and Root, R. B. (1973) Niche, habitat, and ecotope. *The American Naturalist,* 107(955), 321-338.

Wilson, M. J. and McTammany, M. E. (2014) Tributary and mainstem benthic macroinvertebrate communities linked by direct dispersal and indirect habitat alteration. *Hydrobiologia,* 738(1), 75-85.

Winterbourn, M., Rounick, J. and Cowie, B. (1981) Are New Zealand stream ecosystems really different? *New Zealand journal of marine and freshwater research,* 15(3), 321-328.

Woodward, G, and Warren P. (2007) Body Size and Predatory Interactions in Freshwaters: Scaling from Individuals to Communities. In: Hildrew, A.G., Raffaelli, D.G. and Edmonds-Brown, R. (Eds): Body Size: The Structure and Function of Aquatic Ecosystems. Cambridge University Press, Cambridge.

Woodward, G, Ebenman B, Emmerson M, Montoya J. M, Olesen, J. M, Valido, A, Warren P. H (2005) Body Size in Ecological Networks. *Trends in ecology & evolution*, 20:402-409.

Wootton, J. T. and Oemke, M. P (1992) Latitudinal differences in fish community trophic structure, and the role of fish herbivory in a Costa Rican stream. *Environmental Biology of Fishes* 35:311-319.

Wotton, R. S. (1977) The Size of Particles Ingested by Moorland Stream Blackfly Larvae (Simuliidae). *Oikos,* 29(2), 332-335.

Wotton, R. S. and Malmqvist, B. (2001) Feces in Aquatic Ecosystems Feeding animals transform organic matter into fecal pellets, which sink or are transported horizontally

by currents; these fluxes relocate organic matter in aquatic ecosystems. *BioScience,* 51(7), 537-544.

Wowor, D. and Choy S. C. (2001) The freshwater prawns of the genus Macrobrachium Bate, 1868 (Crustacea: Decapoda: Palaemonidae) *from Brunei Darussalam. Raffles Bulletin of Zoology* 49:269-290.

Wowor, D., Cai, Y. and Ng, P. K. (2004) Crustacea: Decapoda, Caridea. *Freshwater Invertebrates of the Malaysian Region. Malaysian Academy of Sciences*, 337-357.

Wright, M. S. and Covich, A.P. (2005) The Effect of Macroinvertebrate Exclusion on Leaf Breakdown Rates in a Tropical Headwater Stream1. *Biotropica* 37: 403-408.

Yang, G. Y. & Dudgeon, D. (2010) Response of grazing impacts of an algivorous fish (Pseudogastromyzon myersi: Balitoridae) to seasonal disturbance in Hong Kong streams. *Freshwater Biology* 55, 411-423.

Young, R. G. and Huryn, A. D. (1999) Effects of land use on stream metabolism and organic matter turnover. *Ecological Applications,* 9(4), 1359-1376.

Yule, C. M. (1996). Spatial distribution of the invertebrate fauna of an aseasonal tropical stream on Bougainville Island, Papua New Guinea. Archiv fur Hydrobiologie. Stuttgart 137:227-249.

Yule, C. M. (2004) Insecta: Diptera. In C. M. Yule & H.-S. Yong (Eds.), *Freshwater invertebrates of the Malaysian region*. Pages 610- 620 *in* C. M. Yule and H.S. Yong, editors, Freshwater invertebrates of the Malaysian region. Academy of Sciences Malaysia, Kuala Lumpur, Malaysia.

Yule, C. M. and Yong, H. S. (2004) *Fresh Water Invertebrates of the Malaysian Region*. Academy of Sciences Malaysia.

Yule, C. M., and Pearson R. G. (1996) Aseasonality of benthic invertebrates in a tropical stream on Bougainville Island, Papua New Guinea. *Archiv für Hydrobiologie* 137: 95-117.

Yule, C. M., Boyero, L. and Marchant, R. (2010) Effects of sediment pollution on food webs in a tropical river (Borneo, Indonesia). *Marine and Freshwater Research,* 61(2), 204-213.

Yule, C. M., Leong, M. Y., Liew, K. C., Ratnarajah, L., Schmidt, K., Wong, H. M., Pearson, R. G. and Boyero, L. (2009) Shredders in Malaysia: abundance and richness are higher in cool upland tropical streams. *Journal of the North American Benthological Society*, *28*(2), 404-415.

Zalewski, M. (2002) Ecohydrology—the use of ecological and hydrological processes for sustainable management of water resources/Ecohydrologie—la prise en compte de processus écologiques et hydrologiques pour la gestion durable des ressources en eau. *Hydrological Sciences Journal,* 47(5), 823-832.

Zettel, H., & Lane, D. J. W. (2010). The creeping water bugs (Insecta: Heteroptera: Naucoridae) of Brunei. Annalen des Naturhistorischen Museums in Wien. Serie B *für Botanik und Zoologie,* 163-171.

Zettel, H., D.J. Lane, and S. Moore. (2008) Aphelocheirus (s. str.) bruneiensis sp. n., a new benthic water bug (Hemiptera: Heteroptera: Aphelocheiridae) from Brunei, and a key to Bornean Aphelocheirus. *Zootaxa*, 1920: 61-68.

Appendices

Appendix for Chapter 4: Eco-Hydromorphic Classification for Understanding Tropical Stream Macroinvertebrate Biodiversity

Appendix Chapter 4a. Density (individual per m^2) and Biomass (AFDM mg m^{-2}) at the Stream 61a. NB: L= larvae

		SP1	SP2	SP3	SR1	SRif2	SRif3
Brachyura							
	Brachyura						
Ephemeroptera							
Leptophlebiidae	Choroterpes	6.52/2.43	6.52/1.41	6.52/2.00		3.26/0.71	
Baetidae	Centroptilum	3.26/0.36	13.03/2.38		6.52/0.62		
	Baetiella						
	Platybaetis						
Heptageniidae	Campsoneuria	3.26/0.64		9.78/7.29		3.26/2.04	
	Epeorus				6.52/7.98		
	Thalerosphyrus	13.03/11.16	29.33/15.06	19.55/2.87		6.52/1.40	3.26/14.26
Caenidae	Caenodes						
Ephemerellidae			3.26/0.77				
Odonata							
Libellulidae	Zygonyx				13.03/4.75		
Gomphidae	Gomphidae					6.52/1.48	9.78/4.57
Platystictidae	Drepanosticta			3.26/3.16			3.26/8.09
	Euphaeid.sp	3.26/5.18	3.26/16.62				
Macromiidae	Macromia						
	Chlorocyphidae						
Perlidae							
Perlidae	Neoperla			3.26/0.64			
Heteroptera							

Nemoiridae	Nemoiridae						
Pleidae	Paraplea	6.52/0.03		3.26/0.02			
Naucoridae	Coptocatus						3.26/23.54
	Thurselinus				6.52/5.02		3.26/49.75
	Gestroiella			3.26/5.16			
	Laccocoris		3.26/3.91	6.52/45.50			
	Coptocatus	6.52/0.46					
Nepidae	Cercotmetus			3.26/593.94			
Megaloptera							
Corydalidae	Protohermes					3.26/20.48	3.26/5.29
Trichoptera							
Brachycentridae	Micrasema						
Calamoceratidae	Ganonema						
Hydropsychidae	Hydropsychidae			3.26/2.95			
	Pseudoleptonema					3.26/13.05	
	Diplectrona					3.26/13.05	3.26/4.59
Helicopsychidae	Helicopsychidae						13.03/1.53
Leptoceridae	Athripsodini						
Calamoceratidae	Anisocentropus					3.26/5.72	
Coleoptera							
Elmidae	Grouvellinus		3.26/0.79		97.75/23.69		55.39/30.6
Elmidae	Elmidae (L)			3.26/0.48			
Psephenidae	Eubrianax	13.03/7.80	26.07/9.43	3.26/2.75			3.26/0.72
Hydrophilidae	Agraphydrus (L)						
Dytiscidae	Neptosternus						6.52/6.76

Eulichadidae	Eulichadidae (L)						
Scirtidae	Scirtidae (L)						3.26/0.14
Diptera							
Athericidae	Asuragina						
	Atrichops						
Simuliidae	Simuliidae						3.26/0.45
Chironomidae	Chironomidae	6.52/0.30	6.52/1.05	13.03/2.85			

Appendix Chapter 4b. Density (individual per m^2) and Biomass (AFDM mg m^{-2}) at the tributary. NB: L= larvae

		TP1	**TP2**	**TP3**	**TR1**	**TR2**	**TRif3**
Brachyura							
	Brachyura		3.26/8.67				
Ephemeroptera							
Leptophlebiidae	Choroterpes	6.52/1.18	6.52/1.31	3.26/0.22			
Baetidae	Centroptilum		3.26/0.48	6.52/3.97		3.26/0.29	3.26/13.16
	Baetiella						3.26/0.29
	Platybaetis				3.26/0.62	3.26/0.29	9.78/4.97
Heptageniidae	Campsoneuria	3.26/0.41	6.52/2.99	22.81/3.08			3.26/0.14
	Epeorus						
	Thalerosphyrus	6.52/2.27	9.78/3.55	9.78/0.46			
Caenidae	Caenodes			3.26/0.46			
Ephemerellidae							
Odonata							
Libellulidae	Zygonyx				6.52/1.41		

Gomphidae	Gomphidae		3.26/5.45	3.26/15.40			6.52/19.40
Platystictidae	Drepanosticta						6.52/1.52
	Euphaeid.sp	6.52/9.23		3.26/0.75			
Macromiidae	Macromia	3.26/3.86	13.03/25.15	19.55/7.21			
	Chlorocyphidae		3.26/2.28	3.26/3.16			
Perlidae							
Perlidae	Neoperla						
Heteroptera							
Nemoiridae	Nemoiridae				3.26/0.13		
Pleidae	Paraplea						
Naucoridae	Coptocatus						
	Thurselinus						
	Gestroiella	3.26/7.19					3.26/5.39
	Laccocoris						
	Coptocatus	3.26/0.04					
Nepidae	Cercotmetus						
Megaloptera							
Corydalidae	Protohermes						3.26/2.41
Trichoptera							
Brachycentridae	Micrasema	3.26/1.59					
Calamoceratidae	Ganonema	3.26/4.01					
Hydropsychidae	Hydropsychidae						
	Pseudoleptonema						3.26/5.50
	Diplectrona						
Helicopsychidae	Helicopsychidae				3.26/0.29	6.52/0.56	

Leptoceridae	Athripsodini			3.26/0.14			
Calamoceratidae	Anisocentropus						
Coleoptera							
Elmidae	Grouvellinus		3.26/0.59		55.39/14.7	9.78/2.58	3.26/1.03
Elmidae	Elmidae (L)						
Psephenidae	Eubrianax	6.52/2.66	19.55/6.99	3.26/2.91			
Hydrophilidae	Agraphydrus (L)						6.52/2.92
Dytiscidae	Neptosternus			3.26/13.50			
Eulichadidae	Eulichadidae (L)						
Scirtidae	Scirtidae (L)						
Diptera							
Athericidae	Asuragina		3.26/6.25				
	Atrichops	3.26/1.90	3.26/0.54	3.26/0.45			
Simuliidae	Simuliidae						
Chironomidae	Chironomidae			3.26/0.07			

Appendix for Chapter 5: Macroinvertebrate Diversity in Pools of Sungai Temburong & Sungai Belalong: A Short Communication

Appendix Chapter 5. Abundance and biomass (AFDM mg) per kick sample at the two rivers, Sungai Temburong (T1-T6) and Sungai Belalong (B1-B6). Larvae = L

		Abbreviation	T1	T2	T3	T4	T5	T6	B1	B2	B3	B4	B5	B6
Ephemeroptera														
Baetidae	Centroptella	Cntrptll		2/0.09	3/0.24	3/0.09	1/0.07	3/0.11						
	Chopralla	Choprall		2/0.24		7/0.49	1/0.09	2/0.07	3/0.24			1/0.05		1/0.14
	Procloeon	Procloen					2/0.07							1/0.14
	Indocloeon	Indoclon										3/0.33		
	Nigrobaetis	Nigrobts			2/0.19	4/0.26	4/0.26	2/0.09						2/0.19
	Labiobaetis	Labiobts										3/0.33	2/0.09	
	Securiops	Seculike												2/0.19
Caenidae	Caenis	Caenis	8/0.5		7/0.53	4/0.17	2/0.08	2/0.24	6/0.63	2/0.16		4/0.44	12/2.01	4/0.46
Ephemerellidae	Hyrtanella	Hyrtanll	1/0.06		1/0.18	2/0.12		2/0.12	5/0.67		3/0.27	4/0.31		
Euthyplociidae	Polyplocia	Polyploc							1/0.87					
Heptageniidae	Compsoneuria	Compsonr	7/4.74			21/4.23	33/5.92	24/5.22	1/0.32		1/0.14	8/3.73	2/3.52	1.68
Leptophlebiidae	Euthraulus	Euthrals	13/2.8	10/1.83	7/1.38	99/11.5	30/4.86	30/3.91	11/1.97		19/3.62	46/8.11	41/7.67	32/4.34
	Thraulus	Thraulus	1/0.04			1/0.04	2/0.16							
Potamanthidae	Potamanthus	Potmnths	3/0.28			4/0.88	4/0.86	7/4.81	4/0.64	6/1.59	8/1.37	3/0.67	85/17.08	
	Rheonanthus sp	Rheosp		1/19.6										
Teloganellidae	Teloganella	Telognll				1/0.26		1/1.57		1/0.04	2/0.44	1/0.26		2/0.7
Teloganodidae	Derlethina	Derlethn				1/0.04	1/0.04		1/0.02			1/0.08	1/0.04	
Odonata														

Calopterygidae	Neurobasis	Neurobss							1/0.1			1/0.03			
Euphaeidae	Euphaeidae	Euphaeid						1/0.68	2/0.02	1/0.68					
Gomphidae	Leptogomphus	Lptgmphs				1/0.01					1/8.9				
Libellulidae	Zygonyx	Zygonyx													1/13.29
Plecoptera															
Perlidae	Neoperla	Neoperla	3/2.91	1/0.81	3/1.17	6/5.87	3/5.02	2/2.84	1/0.99			8/8.75	1/0.64	4/2.01	
Heteroptera															
Naucoridae	Gestroiella	Gestroll	1/0.01												
Veliidae	Rhagovelia	Rhagovel						1/0.68							
Trichoptera															
Ecnomidae	Ecnomidae	Ecnomida								1/0.27	1/0.07	1/0.27		1/0.07	
Calamoceratidae	Anisocentropus	Anscntrp									1/0.31				
Hydropsychidae	Hydromanicus	Hydrmncs	8/3.42		9/1.88	4/2.12	8/1.78	4/2.91	1/0.11			2/0.59			
	Mystacides	Mystacds												1/0	
Leptoceridae	Triplectides	Trplctds									1/0.12				
	Oecetini	Oecetini	3/0.26			2/0.03			2/0.6	1/0	3/0.04	2/0.04	1.72		
Xiphocentronidae	Xiphocentronidae	Xphcntrn	2/0.15	2/0.15	2/0.23		1/0.42								
Lepidoptera															
Crambidae	Elophila	Elophila										2/6.08			
Coleoptera															
Dytiscidae	Neptosternus	NptstrnA			1/0.35						1/0.97				
	Dytisidae(L)	DytisidL				1/0.1						3/0.16			

Elmidae	Dryopomorphus	DrypmrpA		1/0.18					1/0.08					
	ElmidaeGen1(L)	ElmdGn1L	1/0.23	2/0.25	3/0.98	5/1.08	6/1.59	1/0.09	5/1.6	3/0.85	4/1.57	6/1.98	8/1	3/1.12
	ElmidaeGen6(L)	ElmdGn6L	2/0.06	3/0.23		3/0.09	1/0.03	6/0.75	11/0.39	9/0.39	6/0.22	23/1.37	22/1.22	2/0.06
	Grouvellinus	Grovllns									1/0.08		2/0.36	1/0.55
Gyrinidae	Gyrinidae(L)	GyrinidL				1/0.23								
Hydrophilidae	HydrophilidaeGen1 (L)	HyrphG1L		1/0	2/0.2				1/0.03		1/0.1			
Lampyridae	Lampyridae	Lampyrid		1/1.39	1/3.03			1/0.86				1/0.03		
Psephenidae	MicroeubriaL	MicrobrL	1/0.05											
	Eubrianacinae	Eubrincn	1/0.16	1/0.05	1/0.05				1/0.05	1/0.16		3/0.93		
	PsephenoidinaeL	PsphndnL						1/0.05		2/0.07				
Diptera														
Atherceridae	AtherGen1	AtherGn1	2/1.36			2/0.74		1/1					1/0.22	
Ceratopogonidae	Bezzia	Bezzia							1/0.35			1/0.09	1/0.24	
Chioneinae	Chioneinae	Chionein										1/2.39		
Chironomidae	Non predacious chironomidae	Nonchir	8/0.18	7/0.39	8/0.26	13/0.48	17/0.4	8/0.17	5/0.08	1/0.02	11/0.38	25/5.95	6/0.17	11/0.67
	Predacious Chironomidae	PredChir									3/0.21		2/0.17	1/0.04
	ChironGen1	ChirnGn1							1/0.08		1/0.23			

Limoniidae	Hexatoma	Hexatoma	2/3.23	6/17.34	1/0.14	1/0.14	2/0.2	5/5.01		1/4.27
	Eloeophila	Eloeophl				1/0.25				
Simuliidae	Simuliidae	Simuliid							1/0.01	
Tanyderidae	Tanyderidae protanyderus	Tanyderd	1/0.17							

Appendix for Chapter 6: The effect of river-tributary interactions on benthic habitat & macroinvertebrate biodiversity

Appendix Chapter 6a. Abundance and biomass (AFDM mg) per kick sample at the river margin (M)

		1M	**2M**	**3M**	**4M**	**5M**	**6M**	**7M**	**8M**	**9M**	**10M**	**11M**
Collembola			1/0.03				1/0.001					1/0.1
Trombidiformes			1/0.01					4/0.03		1/0.01		
Gastropoda												
Buccinidae	*Clea*	1/0.03							1/0.03			
Oligochaeta	*Oligochaeta*				3/0.58							
Ephemeroptera												
Baetidae	*Centroptella*	3/0.24	7/0.52		1/0.05	2/0.75	4/0.48	2/0.19	25/3.35	20/1.78	4/0.35	2/0.07
	Chopralla	3/0.24						3/0.24	17/1.65	5/0.57		4/0.42
	Procloeon	8/1.04	2/0.19	2/0.23	12/2.15	3/0.43	37/4.95	4/0.32	22/3.12	8/0.84	3/0.18	4/0.42
	Indocloeon	21/2.22	1/0.05	17/2.68	2/0.19	3/0.33					2/0.09	2/0.16
	Nigrobaetis									1/0.14		
	Labiobaetis	1/0.14	2/0.19	3/0.37	4/0.38	1/0.14	3/0.24		4/0.38	1/0.05		
	Baetis								1/0.05			
	Potamic genus											2/0.19
	Baetis G2									1/0.05		
Caenidae	Caenis	34/3.42	18/1.49	12/1.17	6/0.65	23/1.8	22/1.95	20/2.36	33/2.4	93/8.35	9/1.22	1/0.12
Ephemerellidae	*Hyrtanella*											
Euthyplociidae	*Polyplocia*			2/7.4		1/0.87		2/14.43				

Heptageniidae	*Compsoneuria*	20/2.2		5/0.61	11/3.8	11/1.6	15/3.64	3/0.19	2/0.28	25/4.58	1/0.14	2/1.8
	Thalerosphyrus		2/0.09							6/0.92		
Leptophlebiidae	*Euthraulus*	40/8.3	30/4.1	23/2.78	2/0.24	12/1.23	9/1.56	18/2.69	40/6.33	36/5.73	1/0.07	7/0.91
	Thraulus	1/0.52			3/0.44	3/0.68	1/0.12	4/0.32		2/0.16		
	LeptoGen1		1/0.87									
Potamanthidae	*Potamanthus*		3/1.99	2/1.14		1/0.19		2/2.46	1/2.75	1/1.35	2/1.39	
	Rheonanthus					1/3.7		1/11.69		3/46.99		
Teloganellidae	*Teloganella*	6/1.66										
	Dudgeodes						1/0.04	1/0.02				2/0.24
Teloganodidae	*Derlethina*								1/0.46	1/0.26		1/0.46
Odonata												
Aeshnidae	Aeshnidae					1/0.18	1/0.56					
Coenagrionidae	Coenagrionidae		2/0.22									
Calopterygidae	*Neurobasis*							3/4.62				
Euphaeidae	Euphaeidae			1/0.03			2/2.08					
Gomphidae	*Burmagomphus*		1/3.13			1/0.43					2/1	
Gomphidae	*Leptogomphus*								1/0.04			
Libellulidae	*Zygonyx*		1/0.02									
Platycnemididae	Platycnemididae				1/0.01							1/1.02
Platystictidae	Platystictidae							1/0.02				
Plecoptera												
Perlidae	*Neoperla*		1/3.82							1/0.37		1/2.47

Hemiptera												
Corixidae	*Micronecta*								1/0.01			
Veliidae	*Rhagovelia*						1/0.02		2/0.21			7/1.1
Trichoptera												
Calamoceratidae	*Anisocentropus*	2/1.68	2/0.23			1/0.05						5/2.32
Lepidostomatidae	*Lepidostoma*								1/2.66			
	Mystacides	1/0.03	3/0.21	3/0.26		1/0.12		1/0.29	2/0.12	3/0.07	2/0.06	1/0.12
	Triplectides		1/0.12		1/0.01			1/0.12				
	Parasetodes		1/0.19							2/1.67	2/0.23	
	Oecetini	5/0.1	1/0.03					1/0.29	2/0.32		1/0.03	2/0.15
	Nectopsychini											1/0.29
Coleoptera												
Dytiscidae	*Neptosternus* (A)									1/0.97	1/0.97	
Dytiscidae	*Microdytes* (A)						1/0.35					
	Dytisidae (L)								1/0.1	1/0.03		
	ElmidaeGen1 (L)	31/16.67	39/15.84	13/9.17	1/0.45		5/7.41	3/1.07	29/7.26	9/3.31	4/0.85	5/2.49
	ElmidaeGen2 (L)				2/0.13							
	ElmidaeGen5 (L)	2/0.2							1/0.86			
Hydrophilidae	Enochrus (L)						1/0.03					
	Hyrophilidae Gen1 (L)			6/2.71	1/0.25			1/0.25	1/0.49			
	Berous.sp2. L)	1/0.25										
	Eubrianacinae	1/0.16	3/1.02				2/0.75		1/0.16			1/0.05

	Psephenoidinae (L)	6/0.5										
Scirtidae	ScirtidaeGen1 (L)			1/0.03								
Diptera												
Atherceridae	AtherGen1		1/0.22		1/1.32							
	AtherGen2				1/0.22							
Ceratopogonidae	Bezzia			3/2.16			1/0.05		1/0.09			
Chioneinae	Chioneinae	5/2.01		1/0.25								
Chironomidae	Non predacious chironomidae	61/1.49	37/0.82	11/0.32	12/0.32	9/0.33	12/0.47	3/0.11	18/0.66	23/0.91	5/0.1	4/0.23
	Predacious Chironomidae	13/0.63	4/0.2	3/0.26			3/0.21	3/0.2	2/0.12	6/0.26		
	ChironGen1	5/0.77	3/1.98	1/0.04					2/0.31	2/0.91		
	ChironGen3		3/0.8				1/0.08					
Empididae	Hemerodromia		2/0.05									
Ephydridae	Ephydridae			1/0.06								
Limoniidae	Hexatoma		1/4.27	1/25.83			1/0.19		4/1.58	1/1.49	7/1.07	
	Eloeophila			3/39.19								
Muscidae	Muscidae		1/0.06	2/0.12		1/0.04	1/0.06					
Psychodidae	Neotelmatoscopus sp 1	2/0.43									1/0.06	
Simuliidae	Simuliidae		1/0.01	1/0.01				1/0.02		1/0.01	2/0.01	
Tanyderidae	Tanyderidae protanyderus	1/0.01										

Appendix Chapter 6b. Abundance and biomass (AFDM mg) per kick sample at the river-stream interface (I)

		1I	**2I**	**3I**	**4I**	**5I**	**7I**	**8I**	**9I**
Decapoda									
Potamidae	Isolapotamon sp.	1/6.24							
Collembola			3/0.16		1/0.25	1/0.03			
Trombidiformes	Trombidiformes		1/0.01						
Gastropoda									
Buccinidae	Clea	2/7.09				1/0.03			
Clenchiellidae	Clenchiella	1/0				3/0	14/0.01		1/0
Oligochaeta	Oligochaeta				2/2.16				
Tricladida	Tricladida		4/0.24	1/0.02					
Ephemeroptera									
Baetidae	Centroptella				3/0.33	1/0.14		12/0.84	2/0.19
	Chopralla								32/2.92
	Procloeon	2/0.19			5/1.08		1/0.05	18/2.06	12/1.22
	Indocloeon	3/0.24	2/0.09	13/1.42	10/1.05	1/0.32			
	Nigrobaetis								1/0.05
	Labiobaetis	3/0.24	1/0.05		8/1.87			2/0.07	
	Baetis								5/0.52
	Securiops like							4/0.84	
	Baetis sp.					3/0.43		2/0.29	
	Lepto								
Caenidae	Caenis	7/0.35	8/0.61	44/2.99	30/3.18	24/2.46	444/39.24	69/5.26	97/6.53
Ephemerellidae	Hyrtanella	2/1.24							

Euthyplociidae	Polyplocia			3/13.7					
Heptageniidae	Compsoneuria	5/1.08	11/0.91	32/1.72	47/11.62	12/1.86	19/2.77	5/1.75	84/13.64
	Thalerosphyrus							2/0.66	
Leptophlebiidae	Euthraulus	9/0.8	3/0.37	6/1.11	14/4.96	16/3.01	3/0.5	19/4.71	129/23.37
	Thraulus		2/0.4	4/0.79	8/1.59	5/0.76	4/0.2	3/0.6	12/1.69
Potamanthidae	Potamanthus							6/15.41	1/0.12
Teloganellidae	Teloganella				2/0.72				
	Dudgeodes				1/0.12			3/0.22	
Teloganodidae	Derlethina								1.29
Odonata									
Aeshnidae	Aeshnidae						2/28.03		
Calopterygidae	Neurobasis						10/7.45		
Euphaeidae	Euphaeidae	8/0.24			1/0.1		2/2.07		9/9.22
Gomphidae	Burmagomphus						5/10.63	5/1.12	
Gomphidae	Leptogomphus					1/26.23	4/7.7		2/13.42
Gomphidae	Sieboldius						1/45.18		
Libellulidae	Zygonyx	1/0.02							
Platycnemididae	Platycnemididae						6/3.82		
Plecoptera									
Leuctridae	Rhopalopsole								1/0.11
Perlidae	Neoperla	6/4.3				2/1.63		4/1.34	26/16.39
Peltoperlidae	Cryptoperla	3/2.16							
Hemiptera									
Corixidae	Micronecta					1/0		4/0.04	
Helotrephidae	Distotrephes		1/0						
Veliidae	Rhagovelia		6/0.64						

Trichoptera									
Ecnomidae	Ecnomidae	2/0.42		1/0.27				1/0.42	
Eulichadidae	EulichasL	2/10.2							
Calamoceratidae	Anisocentropus	1/0.15	1/0.05	4/2.07	1/1.95	2/0.99	2/1.47		3/0.51
Helicopsychidae	Helicopsyche	1/0.04							
	Macronematinae	3/8.04							
	Diplectrona	17/24.96						1/0.8	
	Hydromanicus	1/0.26							
	Potamyia	1/0.05							
Hydroptilidae	Hellyethira						2/0.23	2/0.07	
	Mystacides	2/0.41	1/0.12	1/0.03	1/0.06	1/0.29		1/0.12	2/0.63
	Parasetodes							11/2.21	
	Oecetini			3/0.33		1/2.71	2/0.06		6/0.25
	Nectopsychini						3/1.01		16/6.17
Philopotamidae	Chimarra	3/2.6							11/2.16
Stenopsychidae	Stenopsychodes	1/0.04							
Curculionidae	Curculionidae(L)		1/1.2						
Coleoptera									
Dytiscidae	NeptosternusA			1/0.35		1/0.61			
Dytiscidae	Microdytes		1/0.35						
Elmidae	ElmidaeGen1L	14/4.89	1/0.09	10/6.42		2/0.6	3/0.55	7/1.98	43/13.59
	ElmidaeGen2L			1/0.06	2/0.3				
	ElmidaeGenus3	1/0.15							
	ElmidaeGen5L	1/0.1							
	Dubiraphia(L)							1/0.1	
	Grouvellinus	1/0.73							

Hydraenidae	Hydraena sp.	2/0.16							
Hydrophilidae	EnochrusL			1/0.1					
	HyrophilidaeGen1L	1/0.25		12/3.1			1/0.1	1/0.49	43/8.33
Hydroscaphidae	HydroscaphaA		1/3.41						
	Eubrianacinae			4/1.64		2/0.54	12/5.11	1/0.16	4/1.21
	PsephenoidinaeL	1/0.1							
Scirtidae	ScirtidaeGen1L	2/0.35	1/0.25	3/7.5					16/3.86
Diptera									
Atherceridae	AtherGen1	1/0.22	1/0.06	1/1					4/0.79
	AtherGen3							1/0.35	
Ceratopogonidae	Bezzia	3/0.88			1/0.09		8/1.31	2/0.48	1/0.16
	Atrichopogon			1/0.03					
Chioneinae	Chioneinae	4/2.24							
Chironomidae	Non predacious chironomidae	20/1.15	8/0.19	38/1.71	34/1.48		265/7.7	27/0.63	53/1.56
	Predacious Chironomidae	1/0.08	1/0.01	2/0.12	11/1.29	9/0.5	13/1.21	8/0.6	5/0.37
	ChironGen1	6/0.96	1/0.08				3/0.54	1/0.23	1/0.15
	ChironGen3			2/0.27					
Limoniidae	Hexatoma	3/3.04			1/9.01		4/10.84	3/5.11	1/1.13
	Eloeophila			1/0.4			3/3.38		
Limnophilinae	Limnophilinae				1.8/2.5				
Muscidae	Muscidae		1/0.02	1/0.02	1/0.06				
Psychodidae	Neotelmatoscopus sp 1	6/0.43	1/0.21		1/0.12				
Psychodidae	Psychodidae						4/0.88	1/0.21	
Simuliidae	Simuliidae	102/2.94							2/0.01
Tanyderidae	Tanyderidae protanyderus						4/0.51		3/0.11

Appendix Chapter 6c. Abundance and biomass (AFDM mg) per kick sample at an upstream pool (U; above a high gradient riffle or waterfall where there is little interaction with the main river)

		1U	2U	3U	4U	5U	6U	7U	8U	9U	10U	11U
Decapoda												
Parathelphusidae	Parathelphusa sp.			1/4.93	1/12.99		1/1.54					
Potamidae	Potamidae		1/1.95									
Potamidae	Isolapotamon sp.	1/1.54	2/5.58									
Collembola			2/0.06	1/0.16							1/0	
Trombidiformes	Trombidiformes				1/0.09				2/0.01			
Gastropoda												
Buccinidae	Clea		2/0.02							1/0.03		
Clenchiellidae	Clenchiella		13/0.01					6/0.01		1/0		2/0.01
Oligochaeta												
Oligochaeta	Oligochaeta				2/0.09			1/0.39				
Tricladida												
Tricladida	Tricladida									1/0.2		
Ephemeroptera												
Baetidae	Centroptella		1/0.05	14/2.64			10/1.34		10/1.23	4/0.38	21/1.61	
	Chopralla					25/2.72			1/0.14	3/0.51		
	Procloeon	1/0.05		16/3.42	3/0.79	3/0.33	1/0.14	1/0.05	1/0.05	2/0.09		
	Indocloeon	2/0.09	19/1.98		4/1.22	1/0.14	17/2.41			1/0.14	5/0.56	
	Nigrobaetis					2/0.19			10/1.41	2/0.14		
	Labiobaetis		4/0.42				4/0.53		6/0.47	3/0.05	14/1.3	3/0.33
	Baetis			1/0.14					2/0.19			

	Liebebiella		1/0.14									
	tiny new genus								3/0.14			
	Baetis sp.									1/0.14		
	Lepto		1/0.14						1/0.05			
Caenidae	Caenis	1.6	1/0.04	7/0.39	34/2.83	33/3.02	24/2.26	200/17.13	24/1.29	109/10.35	1.66	15/2.93
Euthyplociidae	Polyplocia			2/12.36								
Heptageniidae	Compsoneuria	6/0.48	9/0.82	11/0.96	24/1.97	16/2.42	37/5.45	3/0.6	48/7.64	30/5.37	11/1.41	6/1.39
	Thalerosphyrus											7/0.39
Leptophlebiidae	Euthraulus	1/0.12	7/0.76	5/1.66	5/0.92	17/2.47	17/4.05	1/0.12	127/19.69	40/8.53	6/0.95	36/5.98
	Thraulus		1/0.04		16/2.63	6/0.63	12/2.3	2/0.16				1/0.04
Potamanthidae	Potamanthus							3/7.79	6/7.66	2/1.14		
Teloganellidae	Teloganella			1/0.08								
	Dudgeodes					5/0.18						
Teloganodidae	Derlethina							1/0.04	5/0.25	6/0.43	1/0.12	
Odonata												
Aeshnidae	Aeshnidae						1/1.95					
Calopterygidae	Neurobasis					1/0.03			5/0.17	1/3.58		1/1.5
Calopterygidae	Vestalis				1/0.03							
Diphlebidae	Diphlebidae					1/1.02						
Euphaeidae	Euphaeidae	7/8.02	14/8.16	3/4.48		3/8.53		3/1.51	8/8.61		1/0.02	3/16.82
Gomphidae	Heliogomphus									3/0.49		
Gomphidae	Leptogomphus				1/5.53			2/4.26	2/1.57			
Libellulidae	Zygonyx										1/0.02	
Platycnemididae	Platycnemididae				6/2.58	1/2.2		6/3.65		3/1.65		

Platystictidae	Platystictidae					1/2.07						
Plecoptera												
Leuctridae	Rhopalopsole	1/0.21				1/0.11		1/0.34				
Perlidae	Neoperla			4/2.84		4/2.49		2/0.73	15/11.43	16/8.84	4/5.39	7/3.11
Peltoperlidae	Cryptoperla										1/1.54	
Hemiptera												
Corixidae	Micronecta					5/0.03				1/0		
Helotrephidae	Distotrephes		1/0						3/0.05			
Naucoridae	Laccocoris										1/1.99	
Veliidae	Rhagovelia						6/0.84				2/0.33	2/0.63
Trichoptera												
Ecnomidae	Ecnomidae						9/5.22		1/0.15		6/2.28	
Calamoceratidae	Anisocentropus	2/4.61			2/2.17	3/0.76	4/11.49	2/1.22		13/5.78	1/1.37	1/1.95
	Ganonema									4/9.88		
	Diplectrona			1/0.8								
	Hydrospyche										3/2.18	
	Potamyia					4/0.16						
	Ugandatrichia		1/0.42									
Lepidostomatidae	Lepidostoma						1/0.31			1/0.56		
	Mystacides		1/0.12			2/0.15	1/0.12	3/0.26	10/2.74	10/1.73	2/0.32	4/0.37
	Triplectides									3/4.76		
	Parasetodes					2/0.13				1/0.29		
	Oecetini						5/1.33	1/0.12	3/0.18	2/0.58		
	Nectopsychini							6/0.7	6/2.83			
Philopotamidae	Chimarra	1/0.61	2/1.21	1/0.61		2/0.42		3/0.72			2/0.31	
Stenopsychidae	Stenopsychodes					1/0.11						

Xiphocentronidae	Xiphocentronidae				1/0.15							
Lepidoptera												
Crambidae	Elophila								1/0.06			
Crambidae	Paracymoriza	1/0.14										
Curculionidae	Curculionidae(L)											1/1.2
Coleoptera												
Dytiscidae	NeptosternusA								1/0.35			
Dytiscidae	Microdytes									1/0.06		
	DytisidaeL								1/0.03			1/0.06
Elmidae	DryopomorphusA								2/0.36			
	ElmidaeGen1L	5/5.35	10/3.59	7/4.18	1/0.09	2/0.67	1/0.23	5/1.88	60/24.91	24/7.93	2/0.54	12/6.06
	ElmidaeGen2L							4/2.6	1/0		1/0.49	
	ElmidaeGen7L					1/0.1						
Gyrinidae	GyrinidaeL									1/0.23		
	HyrophilidaeGen1L	1/0.1	6/1.36			3/0.6		1/0.25	7/2.56	11/3.01		
	Berosus.sp1.(L)				1/0.1							
Lampyridae	Lampyridae	1/0.1										
Psephenidae	Eubrianacinae		1/0.72		4/1.54	2/0.26	3/0.64	25/7.95	10/2.48	4/0.97	3/1.13	1/0.16
	PsephenoidinaeL			1/0.16								
Scirtidae	ScirtidaeGen1L	1/0.1		1/0.25		8/1.54		1/0.25	1/0.25	2/0.13		
Diptera												
Atherceridae	AtherGen1		1/1					5/8.47	9/2.42	5/4.51	1/0.35	1/0.06
Ceratopogonidae	Bezzia									1/0.09	1/0.16	2/0.25
	Atrichopogon		1/0									
Chioneinae	Chioneinae					3/0.41		2/2.08				
Chironomidae	Non predacious chironomidae	3/0.3	14/0.36	14/0.98	18/0.63	34/0.84	2.46	140/4.89	1/0.02	193/4.61	52/2.53	39/0.93

	Predacious Chironomidae	5/0.44	2/0.23	2/0.12		3/0.18	14/0.85	2/0.1	1.44	5/1.08	2/0.12
	ChironGen1	1/0.08						4/0.4	1/0.04		
	ChironGen3				1/0.34						
Limoniidae	Hexatoma				1/2.39		1/0.4		4/1.22		
	Eloeophila							2/3.4			
Muscidae	Muscidae	1/0.02		1/0.02						1/0.02	1/0.06
Psychodidae	Neotelmatoscopus sp 1		2/1.21							3/0.17	1/2.1
	Neotelmatoscopus sp 2									1/0.12	
Simuliidae	Simuliidae	1/0.04			2/0.07		2/0.05			1.35	1/0.02
Tanyderidae	Tanyderidae protanyderus				4/0.32				1/0.17	3/0.35	

Appendices for Chapter 7: Fluvial biotopes influence macroinvertebrate biodiversity in Southeast Asian tropical streams

Appendix Chapter 7a: Channel characteristics and physical conditions of the biotopes at stream 61a and at the tributary. Type G=gravel, BR= bedrock, B=boulders and C= Cobbles. Discharge calculated from the first riffle in each reach.

		Average depth (m)	Wetted width (m)	Bankful width (m)	Average Velocity (ms^{-1})	Dominant substrate type			
						G	**BR**	**B**	**C**
Sungai Lower Apan: *Discharge = 0.62m^3 Sec^{-1}*									
AP1		0.14	6.62	6.53	- 0.26	70	0	0	30
AP2		0.62	6.61	8.34	- 0.17	95	5	0	0
AP3		1.28	7.17	11.02	- 0.03	90	5	0	5
AP4		0.64	2.97	6.23	- 0.06	60	40	0	0
AP5		0.36	6.24	8.81	- 0.10	50	50	0	0
ARI1		0.21	3.66	19.76	0.65	30	0	10	60
ARI2		0.13	3.54	10.17	0.38	5	80	0	15
AC1		0.04	8.14	3.13	0.60	0	100	0	0
AC2		0.06	2.40	5.19	0.90	0	100	0	0
AC3		0.04	5.85	8.00	0.55	0	100	0	0
AC4		0.06	5.48	8.89	0.35	0	100	0	0
AR1		0.09	8.30	11.21	0.12	10	90	0	0
AWF2	Height ~ 3	0.01	4.22	6.27	1.21	0	100	0	0
AWFT1	Height ~ 3.5	0.01	6.89	9.45	1.38	0	100	0	0
AVERAGE		**0.25**	**5.34**	**9.52**	**0.46**	**31**	**58**	**2**	**8**
Sungai Esu: *Discharge = 0.92m^3 Sec^{-1}*									

EP1		0.49	6.72	10.19	- 0.06	10	5	40	45
EP2		0.58	2.75	3.76	- 0.11	30	60	0	10
EP3		0.71	10.61	10.61	- 0.10	30	60	0	10
EP4		0.40	4.62	4.62	- 0.22	40	0	0	60
EP5		0.80	6.22	6.22	- 0.19	10	90	0	0
ERI1		0.18	3.60	11.80	0.55	20	0	20	60
EC1		0.12	6.37	12.64	0.90	5	0	70	25
EC2		0.07	1.12	4.80	0.86	0	100	0	0
EWF1	Height ~ 4	0.22	3.30	8.37	0.93	0	100	0	0
EWF2	Height ~ 8	0.13	3.45	3.45	1.16	0	100	0	0
AVERAGE		**0.37**	**4.88**	**7.65**	**0.51**	**14.5**	**51.5**	**13**	**21**
Sungai Apan Threelan: : *Discharge = 0.18m³ Sec⁻¹*									
TP1		0.32	3.20	6.70	- 0.07	35	0	5	60
TP2		0.12	2.20	6.30	- 0.09	30	0	0	70
TP3		0.27	8.25	8.25	- 0.09	60	5	0	45
TP4		0.48	7.50	8.60	- 0.12	30	10	10	50
TP5		0.15	3.15	3.70	- 0.12	20	50	0	30
TP6		0.20	2.70	4.50	- 0.06	20	60	0	20
TRI1		0.11	2.80	6.50	0.49	20	0	0	80
TRI2		0.07	3.50	11.20	0.35	30	0	0	70
TR1		0.01	2.50	4.00	0.51	10	80	0	10
TWF1	Height ~ 7	0.02	0.63	4.66	0.93	0	100	0	0
TWF2	Height ~ 5	0.02	4.90	10.20	0.44	0	90	10	0
AVERAGE		**0.16**	**3.76**	**6.78**	**0.3**	**23**	**36**	**2**	**40**

Appendix Chapter 7b. Average density (number of individuals per m^2) and biomass (AFDM m^2) of aquatic insect communities at the 3 study reaches including Lower Apan, Esu and Apan Threelan.

Taxa		Abbreviation	Lower Apan	Esu	Threelan
Decapoda					
Potamidae	Ibanum	Ibanum	0.217/0.381	0/0	0.296/0.303
Trombidiformes					
Torrenticolidae	*Monatractides*	Mntrctds	0/0	1.304/0.003	0.593/0.001
Hygrobatidae	*Hygrobates*	Hygrobts	0.435/0.001	0/0	0.296/0.001
Gastropoda					
	Clea	Clea	0.435/1.731	1.408/3.312	1.334/23.375
	Clenchiella	Clenchll	0.869/0.001	0/0	0/0
Rhynchobdellida					
Glossiphoniidae	Glossiphoniidae	Glssphnd	0/0	0.326/0.011	0/0
Tricladdia					
Tricladdia	Tricladdia	Tricladd	0.435/0.079	0/0	0/0
Ephemeroptera					
Baetidae	*Platybaetis*	Platybts	0.435/0.148	0.978/0.118	0.593/0.248
	Labiobaetis	Labiobts	1.413/0.169	1.082/0.219	1.777/0.143
	Alainites	Alainits	4.453/0.698	1.511/0.611	0/0
	Cloeodes	Cloeodes	0.435/0.074	0/0	0/0
	Nigrobaetis	Nigrobts	0.217/0.026	0/0	0/0
	Centroptella	Cntrptll	2.824/0.544	0.652/0.067	0/0
	Chopralla	Choprall	0.435/0.127	0.978/0.122	0/0
	BaetidaeGenA	BaetA	5.431/0.987	0/0	0.593/0.044
	Gratia	Gratia	0.652/0.213	1.304/0.202	0.593/0.098
	Liebebiella	Liebebll	2.498/0.188	1.63/0.2	0/0
	Asiobaetodes	Asiobtds	2.607/0.239	1.277/0.14	0.889/0.025

	Cloeodes	Cloe1	0.109/0.014	0.313/0.084	0.296/0.012
Heptageniidae	*Compsoneuria*	Compsonr	1.521/0.585	3.584/0.458	2.666/0.905
	Thalerosphyrus	Thlrsphy	1.304/1.019	0.978/1.966	1.185/0.557
Leptophlebiidae	*Euthraulus*	Euthrals	24.546/4.111	45.291/5.168	11.848/1.048
	Thraulus	Thraulus	0/0	0.652/0.01	0/0
	LeptoGen1	LeptoGn1	0/0	0.326/0.152	0/0
Potamanthidae	P*otamanthus*	Potmnths	2.716/3.689	10.754/21.552	6.516/4.437
	Caenis abdita	Caenis	0.435/0.048	11.405/0.792	1.778/0.083
Caenidae	*Caenis*	Caen1	4.345/0.272	11.078/1.032	13.774/0.859
Teloganodidae)	*Dudgeodes*	Dudgeods	1.195/0.136	0.652/0.042	0/0
	Derlethina	Derlethn	3.693/0.694	0/0	0/0
Prosopistomatidae	*Prosopistoma*	Prospstm	0/0	0/0	0.296/0.026
Polymitarcyidae	*Polyplocia*	Polyploc	0/0	0/0	1.185/2.012
Odonata					
Platystictidae	Platystictidae	Pltystct	0.217/0.007	0/0	1.778/1.54
Gomphidae	*Onychogomphus*	Onychgmp	0.217/1.082	0.652/9.349	0.296/0.833
	Burmagomphus	Brmgmphs	0/0	0/0	0.296/0.205
	Leptogomphus	Lptgmphs	0/0	0/0	0.889/5.275
	Sieboldius	Siebolds	0/0	0/0	0.296/2.374
Euphaeidae	Euphaeidae	Euphaeid	0.435/1.651	1.304/1.63	5.036/13.326
Libellulidae	*Zygonyx*	Zygonyx	0.217/0.025	0/0	0.296/1.865
Calopterygidae	*Neurobasis*	Neurobss	0.217/0.02	0/0	0/0
Plecoptera					
Perlidae	*Neoperla*	Neoperla	5.214/2.867	20.202/8.053	17.33/7.353
	Etrocorema	Etrocorm	0/0	2.933/1.21	0.593/0.385
Peltoperlidae	*Cryptoperla*	Cryptprl	0/0	4.236/6.575	0.296/0.011

Leuctridae	*Rhopalopsole*	Rhoplpsl	0.869/0.159	0.978/0.154	0.593/0.112
Heteroptera					
Naucoridae	*Laccocoris*	Laccocrs	0.217/0.015	0/0	0.593/4.155
Corixidae	*Micronecta*	Micronct	0.869/0.003	0/0	0/0
Helotrephidae	*Distotrephes*	Dsttrphs	0.217/0.001	0.652/0.004	0.296/0.002
Veliidae	*Rhagovelia*	Rhagovel	1.521/0.32	5.631/0.879	0/0
Megaloptera					
Corydalidae	*Protohermes*	Prothrms	0/0	0.326/0.023	0/0
Trichoptera					
Hydroptilidae	*Hydroptila*	Hydroptl	0/0	0.978/0.01	0/0
	Ugandatrichia	Ugndtrch	0/0	0.652/0.032	0.741/0.004
	Orthotrichia	Orthtrch	0.652/0.013	0/0	0/0
Philopotamidae	*Chimarra*	Chimarra	1.195/0.248	4.563/1.028	9.775/2.111
Ecnomidae		Ecnomida	0/0	4.888/0.552	1.185/0.213
Xiphocentronidae		Xphcntrn	0.109/0.026	0.652/0.271	1.185/0.045
Hydrobiosidae	*Apsilochorema*	Apslchrm	0/0	0.326/0.55	0/0
Polycentropodidae		Plycntrp	0/0	0/0	0.296/1.949
Stenopsychidae	*Stenopsychodes*	Stnpsych	0/0	0/0	0.296/0.111
Diplectroninae	*Diplectrona*	Diplctrn	1.086/5.915	0.326/0.741	1.777/2.591
Hydropsychidae	*Hydromanicus*	Hydrmncs	2.498/14.379	3.585/18.506	7.11/17.66
	Macrostemum	Macrstmm	0/0	1.63/5.423	3.259/2.876
	Hydrospyche	Hydrspyc	0.652/4.329	1.082/7.905	0.593/1.373
	Cheumatopsyche	Chmtpsyc	0/0	0.326/0.334	1.185/0.997
	Potamyia	Potamyia	0.326/0.212	6.192/4.334	19.255/10.038
	Polymorphanisus	Plymrphn	0/0	0/0	0.296/6.535
Leptoceridae	*Lepidostoma*	Lepidstm	0/0	0.326/0.267	0/0

	Oecetini	Oecetini	1.413/0.327	3.585/0.497	2.37/0.199
	Athripsodini	Athrpsdn	0/0	0/0	0.296/0.051
	Nectopsychini	Nctpsych	0/0	0/0	0.296/0.078
Calamoceratidae	*Anisocentropus*	Anscntrp	0.652/0.338	0.978/1.306	6.814/6.767
	Ganonema	Ganonema	0/0	0/0	0.593/2.372
Lepidoptera					
Crambidae	*Eoophyla*	Eoophyla	0.652/0.163	0.652/0.291	1.185/0.364
	Elophila	Elophila	0.109/0.011	0/0	0/0
	Paracymoriza	Parcymrz	0.217/1.479	0/0	0.296/1.306
Coleoptera					
Dytiscidae	*Neptosternus* (A)	NptstrnA	1.303/0.411	0/0	1.481/0.651
	Dytisidae (L)	DytisidL	0.435/0.022	2.607/0.192	1.185/0.065
Gyrinidae	Gyrinidae (L)	GyrinidL	0/0	0.326/0.219	0.593/4.656
Hydrophilidae	*Pelthydrus* (A)	PlthydrA	0/0	0.978/0.641	1.777/0.56
	Enochrus (L)	EnochrsL	0.869/0.289	5.214/1.662	2.666/0.655
	HyrophilidaeGen2 (L)	HyrphG2L	0.217/0.02	0/0	0.296/0.027
	HyrophilidaeGen1 (L)	HyrphG1L	0/0	4.888/1.112	0.889/0.197
Scirtidae	ScirtidaeGen1 (L)	ScrtdG1L	1.63/0.209	5.214/0.752	5.925/0.73
	ScirtidaeGen2 (L)	ScrtdG2L	0/0	0.652/0.078	0/0
Psephenidae	*Schinostethus* (L)	SchnsttL	0/0	0/0	0.593/0.29
	Microeubria (L)	MicrobrL	0/0	0/0	0.296/0.032
	EubrianaxGen1 (L)	EbrnxG1L	0.217/0.128	2.282/1.713	2.666/0.691
	Psephenoidinae (L)	PsphndnL	0/0	0/0	1.037/0.043
	EubrianaxGen2	EbrnxGn2	0/0	0.652/0.06	0/0
Elmidae	*Stenelmis* (A)	StenlmsA	1.087/0.309	3.585/0.963	2.963/0.561
	Dryopomorphus (A)	DrypmrpA	0/0	0.652/0.849	0/0

	ElmidaeGen1 (L)	ElmdGn1L	18.681/5.387	94.819/28.482	50.505/10.1
	ElmidaeGen2 (L)	ElmdGn2L	0/0	0.326/0.254	0/0
	ElmidaeGen3 (L)	ElmdGn3L	0/0	0.652/0.088	1.185/0.073
	ElmidaeGen4 (L)	ElmdGn4L	0/0	0.326/0.144	0/0
	ElmidaeGen5 (L)	ElmdGn5L	0.109/0.016	3.259/0.176	0/0
	ElmidaeGen6 (L)	ElmdGn6L	0.109/0.024	0/0	0/0
	ElmidaeGen7 (L)	ElmdGn7L	0/0	0/0	0.593/0.03
Diptera					
Tipulidae. Limoniidae	*Limnophilinae*	Limnphln	0.217/0.578	3.259/25.845	5.035/12.076
	Chioneinae	Chionein	0.652/0.875	2.607/4.151	5.925/4.154
	Gonomyia	Gonomyia	1.955/0.919	0.326/0.024	0/0
	Antocha	Antocha	0.109/0.03	1.304/0.349	0/0
	Geranomyia	Geranomy	0/0	0/0	1.185/0.805
Psychodidae	*Neotelmatoscopus*	Ntlmtscp	2.607/0.123	15.628/0.652	10.221/0.645
	Psychodidae	Psychodd	0/0	0/0	0.593/1.964
Ceratopogonidae	*Bezzia*	Bezzia	0.217/0.03	0/0	0.889/0.031
Simuliidae	Simuliidae	Simuliid	108.505/4.645	5.318/0.136	9.776/0.163
Chironomidae	Non predacious chironomidae	Nonchir	13.685/0.254	41.381/1.441	45.915/0.905
	Predacious Chironomidae	PredChir	4.563/0.224	42.894/2.439	23.995/1.055
	ChironGen1	ChirnGn1	0.217/0.009	0/0	0.296/0.036
	ChironGen2	ChirnGn2	0/0	0.326/0.005	0.593/0.013
	ChironGen3	ChirnGn3	0/0	3.91/1.428	0.593/0.198
Athericidae	AtherGen1	AtherGn1	0/0	0/0	0.889/0.229
	AtherGen2	AtherGn2	0/0	0/0	1.481/0.266
	AtherGen3	AtherGn3	0/0	0/0	0.296/0.173
	Ephydridae	Ephydrid	0/0	0/0	0.296/0.006

Blephariceridae	BlephGen1	BlephGn1	0.761/0.653	1.629/1.167	0.889/0.455
	BlephGen3	BlephGn3	0/0	0.326/0.078	0.296/0.013
	BlephGen4	BlephGn4	0.543/0.225	0/0	0/0
Muscidae	Muscidae	Muscidae	0/0	0.652/0.477	0.889/0.032

Appendices for Chapter 8: Benthic Community Structure & Ecosystem Functions in Above-and Below-Waterfall Pools in Borneo

Appendix Chapter 8a. Total density (number of individuals per m^2) and biomass (AFDM m^2) of macroinvertebrates at Apan Threelan. (A) refers to adults and (L) refers to larvae. Pools in bold (TP3 & TP4) are the experimental pools.

		Threelan	TP1	TP2	**TP3**	**TP4**	TP5	TP6
Decapoda								
Potamidae	Ibanum	0.54/0.56	0/0	3.26/3.33	0/0	0/0	0/0	0/0
Trombidiformes								
Torrenticolidae	*Monatractides*	0.54/0.001	0/0	0/0	3.26/0.004	0/0	0/0	0/0
Hygrobatidae	*Hygrobates*	0.54/0.001	0/0	0/0	3.26/0.01	0/0	0/0	0/0
Gastropoda								
	Clea	0.54/41.93	3.26/251.55	0/0	0/0	0/0	0/0	0/0
Ephemeroptera								
Baetidae	*Platybaetis*	1.09/0.46	0/0	0/0	0/0	0/0	6.52/2.73	0/0
	Cloeodes	0.54/0.02	0/0	0/0	0/0	0/0	3.26/0.13	0/0
Heptageniidae	*Compsoneuria*	1.63/0.29	0/0	0/0	0/0	0/0	9.78/1.77	0/0
Leptophlebiidae	*Euthraulus*	12.49/1.34	3.26/1.52	13.03/0.94	19.55/3.37	13.03/0.81	26.07/1.39	0/0
Potamanthidae	*Potamanthus*	11.95/8.14	0/0	13.03/15.36	13.03/4.46	13.03/13.07	22.81/15.71	9.78/0.21
Caenidae	*Caenis abdita*	1.63/0.13	0/0	0/0	0/0	9.78/0.76	0/0	0/0
	Caenis sp	22.81/1.56	13.03/1.19	3.26/0.36	61.91/2.91	16.29/1.38	32.58/2.27	9.78/1.23
Prosopistomatidae	*Prosopistoma*	0.54/0.05	3.26/0.29	0/0	0/0	0/0	0/0	0/0
Polymitarcyidae	*Polyplocia*	1.09/3.42	0/0	0/0	3.26/12.47	3.26/8.05	0/0	0/0
Odonata								
Platystictidae	Platystictidae	0.54/0.11	0/0	3.26/0.66	0/0	0/0	0/0	0/0

Gomphidae	*Burmagomphus*	0.54/0.38	0/0	0/0	0/0	0/0	3.26/2.26	0/0
	Leptogomphus	1.63/9.67	0/0	3.26/47.15	0/0	3.26/1.72	0/0	3.26/9.16
	Sieboldius	0.54/4.35	0/0	0/0	0/0	0/0	0/0	3.26/26.11
Euphaeidae	Euphaeidae	7.06/18.38	0/0	22.81/64.29	9.78/28.76	6.52/11.14	0/0	3.26/6.06
Libellulidae	*Zygonyx*	0.54/3.42	0/0	3.26/20.51	0/0	0/0	0/0	0/0
Plecoptera								
Perlidae	*Neoperla*	14.12/4.7	9.78/1.72	22.81/10.14	39.1/7.88	9.78/7.96	0/0	3.26/0.49
	Etrocorema	1.09/0.71	0/0	0/0	0/0	0/0	6.52/4.24	0/0
Leuctridae	*Rhopalopsole*	1.09/0.2	3.26/1.00	0/0	0/0	3.26/0.23	0/0	0/0
Heteroptera								
Naucoridae	*Laccocoris*	1.09/7.62	0/0	0/0	0/0	0/0	3.26/31.4	3.26/14.3
Helotrephidae	*Distotrephes*	0.54/0.003	3.26/0.02	0/0	0/0	0/0	0/0	0/0
Trichoptera								
Philopotamidae	*Chimarra*	1.09/0.11	0/0	6.52/0.67	0/0	0/0	0/0	0/0
Ecnomidae		1.09/0.28	6.52/1.67	0/0	0/0	0/0	0/0	0/0
Stenopsychidae	*Stenopsychodes*	0.54/0.2	0/0	0/0	3.26/1.22	0/0	0/0	0/0
Diplectroninae	*Diplectrona*	1.09/0.13	0/0	0/0	6.52/0.81	0/0	0/0	0/0
Hydropsychidae	*Polymorphanisus*	0.54/11.98	0/0	3.26/71.89	0/0	0/0	0/0	0/0
Leptoceridae	*Oecetini*	4.34/0.37	3.26/0.34	13.03/1.33	6.52/0.43	0/0	0/0	3.26/0.09
	Nectopsychini	0.54/0.14	0/0	3.26/0.86	0/0	0/0	0/0	0/0
Calamoceratidae	*Anisocentropus*	11.95/12.36	13.03/8.63	9.78/13.9	6.52/5.66	16.29/17.71	3.26/1.65	22.81/26.63
	Ganonema	1.09/4.35	0/0	0/0	0/0	6.52/26.09	0/0	0/0
Coleoptera								

Dytiscidae	*Neptosternus Gen A*	2.72/1.19	13.03/4.33	3.26/2.83	0/0	0/0	0/0	0/0
	Dytisidae (L)	1.63/0.11	0/0	0/0	0/0	9.78/0.64	0/0	0/0
Gyrinidae	*Gyrinidae (L)*	0.54/7.98	0/0	0/0	0/0	3.26/47.88	0/0	0/0
Hydrophilidae	*Enochrus (L)*	3.8/1.2	0/0	13.03/4.65	6.52/1.52	0/0	0/0	3.26/1.04
	Hyrophilidae Gen 2 (L)	0.54/0.05	0/0	0/0	0/0	0/0	3.26/0.3	0/0
	Hyrophilidae Gen 1 (L)	0.54/0.12	0/0	0/0	0/0	3.26/0.72	0/0	0/0
Scirtidae	*Scirtidae Gen1 (L)*	2.17/0.58	6.52/2.89	3.26/0.3	0/0	3.26/0.3	0/0	0/0
Psephenidae	*Eubrianax Gen1 (L)*	2.17/0.44	3.26/1.37	9.78/1.3	0/0	0/0	0/0	0/0
	Psephenoidinae (L)	1.63/0.06	0/0	6.52/0.19	0/0	0/0	3.26/0.19	0/0
Elmidae	*Stenelmis (A)*	1.63/0.42	3.26/0.98	3.26/0.98	0/0	0/0	0/0	3.26/0.53
	Elmidae Gen1 (L)	54.31/12.34	61.91/17.81	78.2/17.14	42.36/5.56	13.03/3.96	29.33/5.27	101.01/24.32
	Elmidae Gen3 (L)	0.54/0.02	0/0	0/0	0/0	0/0	3.26/0.14	0/0
	Elmidae Gen7 (L)	1.09/0.06	0/0	3.26/0.3	3.26/0.03	0/0	0/0	0/0
Diptera								
Tipulidae. Limoniidae	Limnophilinae	4.89/12.05	3.26/41.8	16.29/16.85	3.26/12.93	3.26/0.5	3.26/0.24	0/0
	Chioneinae	5.97/5.29	3.26/3.02	19.55/20.67	6.52/2.49	6.52/5.54	0/0	0/0
Psychodidae	*Neotelmatoscopus*	1.63/0.12	0/0	9.78/0.71	0/0	0/0	0/0	0/0
	Psychodidae	1.09/3.6	0/0	0/0	6.52/21.6	0/0	0/0	0/0
Ceratopogonidae	*Bezzia*	1.63/0.06	0/0	0/0	3.26/0.16	3.26/0.13	3.26/0.05	0/0
Simuliidae	Simuliidae	1.63/0.02	3.26/0.03	3.26/0.05	0/0	3.26/0.07	0/0	0/0
Chironomidae	Non predacious chironomidae	66.25/1.43	19.55/0.66	107.53/2.47	169.44/2.13	39.1/0.99	52.13/2.11	9.78/0.23
	Predacious Chironomidae	29.33/1.48	29.33/1.66	29.33/1.83	58.65/2.27	19.55/1.57	22.81/0.75	16.29/0.83

	ChironGen1	0.54/0.07	0/0	0/0	0/0	0/0	3.26/0.4	0/0
Athericidae	AtherGen1	1.63/0.42	0/0	6.52/2.07	0/0	0/0	3.26/0.45	0/0
	AtherGen2	2.72/0.49	0/0	0/0	16.29/2.93	0/0	0/0	0/0
	AtherGen3	0.54/0.32	3.26/1.9	0/0	0/0	0/0	0/0	0/0
	Ephydridae	0.54/0.01	0/0	0/0	3.26/0.07	0/0	0/0	0/0
Muscidae	Muscidae	1.09/0.05	0/0	0/0	0/0	3.26/0.21	3.26/0.07	0/0

Appendix Chapter 8b. Total density (number of individuals per m^2) and biomass (AFDM m^2) of macroinvertebrates at Esu. A) refers to adults and (L) refers to larvae. Pools in bold (EP2 & EP3) are the experimental pools.

Taxa		Esu	EP1	**EP2**	**EP3**	EP4	EP5
Trombidiformes							
Torrenticolidae	*Monatractides*	1.96/0.004	9.78/0.02	0/0	0/0	0/0	0/0
Gastropoda							
	Clea	0.65/0.004	0/0	0/0	0/0	3.26/0.02	0/0
Rhynchobdellida							
Glossiphoniidae	Glossiphoniidae	0.65/0.02	0/0	3.26/0.11	0/0	0/0	0/0
Ephemeroptera							
Baetidae	*Labiobaetis*	0.65/0.19	0/0	0/0	3.26/0.95	0/0	0/0
	Alainites	2.61/1.2	0/0	0/0	0/0	13.03/6.01	0/0
	Chopralla	1.96/0.24	0/0	9.78/1.22	0/0	0/0	0/0
Heptageniidae	*Compsoneuria*	3.91/0.34	6.52/0.53	13.03/1.18	0/0	0/0	0/0
Leptophlebiidae	*Euthraulus*	49.53/6.46	45.62/6.62	91.23/8.79	74.94/10.38	32.58/6.41	3.26/0.11
Potamanthidae	*Potamanthus*	19.55/42.85	16.29/12.03	9.78/1.73	39.1/90.41	26.07/86.1	6.52/23.97
Caenidae	*Caenis abdita*	21.51/1.53	0/0	0/0	13.03/0.61	87.98/6.3	6.52/0.72
	Caenis sp.	16.29/1.64	9.78/0.6	3.26/0.36	39.1/4.96	16.29/1.35	13.03/0.92
Odonata							
Gomphidae	*Onychogomphus*	0.65/0.9	0/0	3.26/4.48	0/0	0/0	0/0
Euphaeidae	Euphaeidae	0.65/2.67	0/0	3.26/13.35	0/0	0/0	0/0
Plecoptera							
Perlidae	*Neoperla*	28.02/11.18	19.55/8.33	0/0	45.62/10.22	61.91/31.3	13.03/6.04
	Etrocorema	4.56/0.96	0/0	22.81/4.78	0/0	0/0	0/0
Peltoperlidae	*Cryptoperla*	0.65/0.56	0/0	0/0	3.26/2.80	0/0	0/0
Leuctridae	*Rhopalopsole*	1.96/0.31	0/0	0/0	0/0	9.78/1.54	0/0
Heteroptera							

Helotrephidae	*Distotrephes*	1.3/0.01	3.26/0.01	0/0	0/0	3.26/0.03	0/0
Veliidae	*Rhagovelia*	1.3/0.58	0/0	3.26/0.92	0/0	0/0	3.26/1.98
Megaloptera							
Corydalidae	*Protohermes*	0.65/0.05	0/0	0/0	3.26/0.23	0/0	0/0
Trichoptera							
Philopotamidae	*Chimarra*	7.17/1.64	0/0	0/0	3.26/1.49	3.26/0.78	29.33/5.93
Ecnomidae		8.47/0.92	0/0	0/0	0/0	35.84/3.04	6.52/1.56
Xiphocentronidae		0.65/0.3	0/0	0/0	3.26/1.49	0/0	0/0
Hydrobiosidae	*Apsilochorema*	0.65/1.1	0/0	0/0	0/0	3.26/5.50	0/0
Diplectroninae	*Diplectrona*	0.65/1.48	0/0	0/0	3.26/7.41	0/0	0/0
Hydropsychidae	*Macrostemum*	3.26/10.85	0/0	0/0	6.52/18.19	6.52/31.06	3.26/4.98
Leptoceridae	*Oecetini*	3.91/0.53	6.52/0.43	9.78/1.88	3.26/0.34	0/0	0/0
Calamoceratidae	*Anisocentropus*	1.3/1.05	0/0	0/0	3.26/4.82	0/0	3.26/0.43
Coleoptera							
Dytiscidae	Dytisidae (L)	5.21/0.38	0/0	0/0	3.26/0.3	19.55/1.32	3.26/0.3
Hydrophilidae	*Pelthydrus (A)*	0.65/0.36	0/0	0/0	0/0	0/0	3.26/1.79
	Enochrus (L)	8.47/2.73	0/0	0/0	16.29/5.38	0/0	26.07/8.27
	Hyrophilidae Gen1 (L)	9.78/2.22	9.78/1.19	13.03/2.47	0/0	26.07/7.46	0/0
Scirtidae	*Scirtidae Gen1 (L)*	5.21/0.70	3.26/0.17	3.26/0.30	3.26/0.48	0/0	16.29/2.53
Psephenidae	*Eubrianax Gen1 (L)*	3.26/1.26	0/0	9.78/4.56	6.52/1.72	0/0	0/0
Elmidae	*Stenelmis (A)*	6.52/1.82	3.26/0.98	19.55/5.63	6.52/1.51	3.26/0.98	0/0
	Elmidae Gen1 (L)	149.23/42.35	143.37/40.53	114.04/33.15	306.29/77.1	123.82/49.3	58.65/11.66
	Elmidae Gen2 (L)	0.65/0.51	0/0	0/0	3.26/2.54	0/0	0/0
	Elmidae Gen3 (L)	1.3/0.18	0/0	0/0	3.26/0.44	3.26/0.44	0/0
	Elmidae Gen5 (L)	0.65/0.14	0/0	0/0	0/0	0/0	3.26/0.72
Diptera							

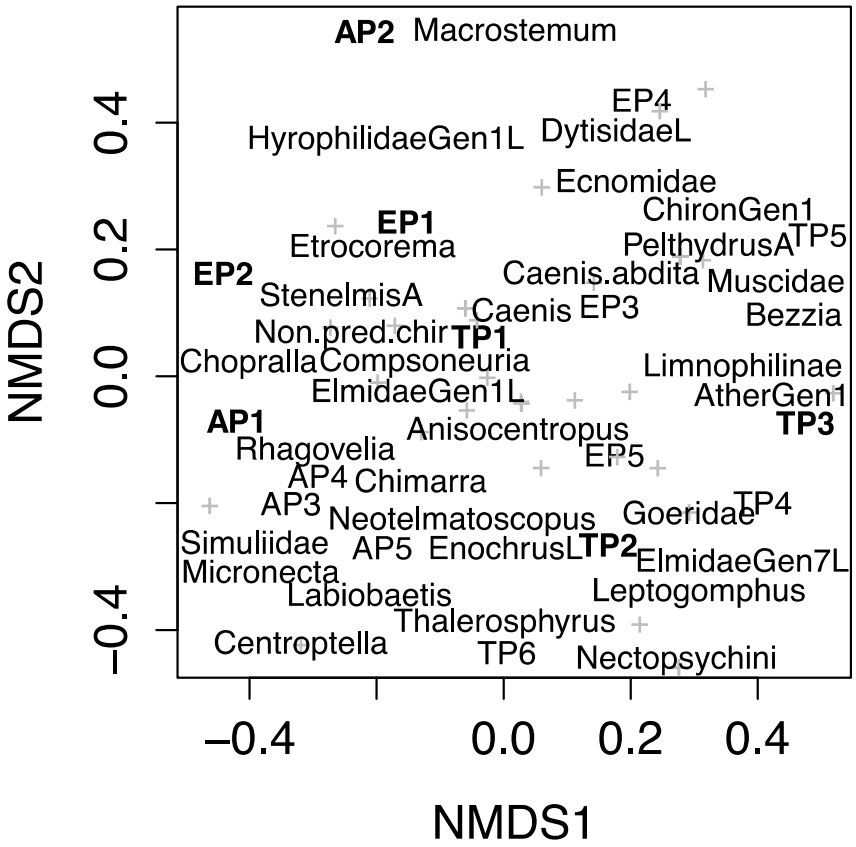
Tipulidae. Limoniidae	Limnophilinae	5.87/51.08	0/0	0/0	9.78/15.57	6.52/57.47	13.03/182.39
	Chioneinae	5.21/8.3	0/0	0/0	0/0	9.78/20.52	16.29/20.99
	Antocha	1.96/0.63	0/0	0/0	0/0	9.78/3.14	0/0
Psychodidae	*Neotelmatoscopus*	0.65/0.04	0/0	3.26/0.2	0/0	0/0	0/0
Simuliidae	Simuliidae	1.3/0.03	0/0	3.26/0.07	0/0	0/0	3.26/0.07
Chironomidae	Non predacious chironomidae	60.61/2.5	0/0	162.92/4.97	0/0	97.75/6.59	42.36/0.95
	Predacious Chironomidae	70.38/4.22	9.78/0.48	81.46/4.72	74.94/7.3	159.66/7.4	26.07/1.21
	Chiron Gen2	0.65/0.01	0/0	0/0	3.26/0.05	0/0	0/0
	Chiron Gen3	7.82/2.86	0/0	0/0	3.26/0.99	16.29/7.5	19.55/5.79
Muscidae	Muscidae	0.65/0.95	0/0	0/0	3.26/4.77	0/0	0/0

Appendix Chapter 8c. Total density (number of individuals per m^2) and biomass (AFDM m^2) of macroinvertebrates at Lower Apan. A) refers to adults and (L) refers to larvae. Pools in bold (AP2 & AP3) are the experimental pools.

Taxa		Lower Apan	AP1	**AP2**	**AP3**	AP4	AP5
Decapoda							
Potamidae	Ibanum	0.65/1.14	3.26/5.72	0/0	0/0	0/0	0/0
Gastropoda							
	Clea	0.65/0.15	0/0	3.26/0.77	0/0	0/0	0/0
Tricladdia							
Tricladdia	*Tricladdia*	1.3/0.24	0/0	0/0	6.52/1.19	0/0	0/0

Ephemeroptera							
Baetidae	*Labiobaetis*	3.26/0.35	0/0	0/0	3.26/0.65	6.52/0.84	6.52/0.27
	Alainites	0.65/0.19	3.26/0.95	0/0	0/0	0/0	0/0
	Centroptella	8.47/1.63	0/0	0/0	0/0	0/0	42.36/8.16
	Chopralla	1.3/0.38	0/0	0/0	0/0	0/0	6.52/1.90
Heptageniidae	*Compsoneuria*	1.96/0.67	0/0	0/0	0/0	3.26/3.11	6.52/0.25
	Thalerosphyrus	1.30/0.11	0/0	0/0	0/0	0/0	6.52/0.53
Leptophlebiidae	*Euthraulus*	56.7/10.44	45.62/7.12	35.84/3.84	42.36/5.58	39.1/6.72	120.56/28.95
Potamanthidae	*Potamanthus*	5.87/9.94	0/0	0/0	16.29/21.97	9.78/26.21	3.26/1.52
Caenidae	*Caenis abdita*	0.65/0.08	0/0	0/0	3.26/0.39	0/0	0/0
	Caenis sp.	11.73/0.72	0/0	26.07/1.22	0/0	19.55/1.46	13.03/0.92
Odonata							
Platystictidae	Platystictidae	0.65/0.02	0/0	3.26/0.10	0/0	0/0	0/0
Gomphidae	*Onychogomphus*	0.65/3.25	0/0	3.26/16.23	0/0	0/0	0/0
Euphaeidae	Euphaeidae	0.65/4.07	3.26/20.37	0/0	0/0	0/0	0/0
Calopterygidae	*Neurobasis*	0.65/0.06	0/0	3.26/0.30	0/0	0/0	0/0
Plecoptera							
Perlidae	*Neoperla*	9.12/5.71	9.78/6.44	3.26/1.88	3.26/8.17	6.52/6.66	22.81/5.37
Leuctridae	*Rhopalopsole*	0.65/0.16	3.26/0.79	0/0	0/0	0/0	0/0
Heteroptera							
Naucoridae	*Laccocoris*	0.65/0.05	0/0	0/0	0/0	0/0	3.26/0.23
Corixidae	*Micronecta*	1.96/0.01	0/0	0/0	3.26/0.02	6.52/0.02	0/0
Helotrephidae	*Distotrephes*	0.65/0.002	0/0	0/0	0/0	3.26/0.01	0/0
Veliidae	*Rhagovelia*	3.26/0.68	3.26/0.57	0/0	3.26/0.92	6.52/0.97	3.26/0.92
Trichoptera							
Philopotamidae	*Chimarra*	2.61/0.62	3.26/0.78	0/0	6.52/1.1	3.26/1.22	0/0

Leptoceridae	*Oecetini*	2.61/0.94	3.26/1.25	0/0	3.26/1.75	3.26/0.86	3.26/0.86
Calamoceratidae	*Anisocentropus*	1.96/1.01	0/0	3.26/0.15	3.26/4.01	3.26/0.91	0/0
Coleoptera							
Dytiscidae	Dytisidae (L)	0.65/0.01	0/0	3.26/0.03	0/0	0/0	0/0
Hydrophilidae	*Enochrus (L)*	1.30/0.43	3.26/1.44	0/0	3.26/0.72	0/0	0/0
	Hyrophilidae Gen2 (L)	0.65/0.06	0/0	0/0	0/0	0/0	3.26/0.30
Scirtidae	*Scirtidae Gen1 (L)*	1.96/0.31	0/0	0/0	0/0	6.52/1.45	3.26/0.08
Elmidae	*Stenelmis (A)*	1.30/0.39	3.26/0.98	3.26/0.98	0/0	0/0	0/0
	Elmidae Gen1 (L)	37.81/11.31	74.94/23.11	13.03/4.25	42.36/13.76	19.55/7.05	39.1/8.40
Diptera							
Tipulidae. Limoniidae	*Chioneinae*	0.65/1.73	0/0	0/0	3.26/8.67	0/0	0/0
Simuliidae	Simuliidae	15.64/0.54	6.52/0.15	0/0	39.1/1.43	29.33/1.06	3.26/0.07
Chironomidae	Non predacious chironomidae	14.99/0.3	6.52/0.29	19.55/0.40	22.81/0.25	13.03/0.26	13.03/0.30
	Predacious Chironomidae	9.12/0.42	3.26/0.08	19.55/0.77	9.78/0.79	3.26/0.12	9.78/0.37



Appendix Chapter 8d. NMDS ordination of macroinvertebrate abundance among sampled pools. The first letter represents the stream; T, Sungai Apan Threelan; A, Sungai Lower Apan; E, Sungai Esu. Second letter is P to represent pool and the number represents the pool at that study stream. The sites in bold are the pools situated below the waterfall. Stress = >0.2

Appendix Chapter 8e. Total relative abundance and biomass of shrimp in pools above and below the study sites Apan Threelan, Esu and Lower Apan. Pools in bold are the experimental pools.

Site	Abundance	Biomass
Threelan *(Below)*	1	75.82
Threelan *(Above)*	84	26532.55
Esu *(Below)*	0	0
Esu *(Above)*	53	12185.87
Lower Apan *(Below)*	1	198.74
Lower Apan *(Above)*	1	8.21
TP1	0	0
TP2	1	75.82
TP3	0	0
TP4	24	3914.30
TP5	7	2964.40
TP6	53	19653.85
EP1	0	0
EP2	0	0
EP3	34	6413.47
EP4	12	2172.16
EP5	7	3600.24
AP1	1	198.74
AP2	0	0
AP3	0	0
AP4	1	8.21
AP5	0	0

Appendix Chapter 8f. Total density of fish at the study sites (number of individuals per m^2)

Family	Genus	Threelan		Esu		Lower Apan		Baki	
		Below (TP3)	**Above (TP4)**	**Below (EP2)**	**Above (EP3)**	**Below (AP2)**	**Above (AP3)**	**Below (BP3)**	**Above (BP4)**
Gastromyzon		0	0	0	0.02	0	0	0.009	0
Cyprinidae	Nematabramis steindachneri	0.01	0	0.16	0	0.27	0.02	0	0
Cyprinidae	Tor douronensis	0.07	0	0.03	0	0	0	0	0
Cyprinidae	Rasbora argyrotaenia	0	0	0	0	0.30	0.04	0	0
Cyprinidae	Hampala bimaculata	0	0	0	0	0.09	0	0	0
Cyprinidae	Rasbora Sumatrana	0	0	0	0	0.03	0	0	0

Appendix for Chapter 9: Macroinvertebrate trophic structure on Waterfalls in Borneo

Appendix Chapter 9. Carbon and nitrogen isotopic values of basal food sources and consumers at the 12 waterfalls

Waterfall	Periphyton		Leaf litter	
	$\delta^{13}C$	**$\delta^{15}N$**	**$\delta^{13}C$**	**$\delta^{15}N$**
One	-32.12	0.64	-32.48	-0.37
	-31.99	0.8	-32.29	-0.24
	-32.51	1.99	-32.38	-0.59
	-32.15	1.19		
	-32.19	1.47		
Two	-27.19	0.66	-32.21	-1.06
	-27.1	0.66	-32.17	-1.24
	-27.21	0.15	-32.07	-1.39
	-26.39	0.3		
	-26.47	0.73		
Three	-25.09	1.2	-32.66	-1.8
	-26.33	1.31	-32.62	-1.49
	-25.64	1.14	-32.22	-2.43
Four	-30.66	1.18	-35.42	0.52
	-30.31	1.17	-34.78	0.41
	-30.56	1.39	-35.16	0.41
	-30.38	0.95		
Five	-32.85	0.67	-32.67	-0.5
	-32.76	0.59	-32.61	-0.58
	-32.7	0.49	-32.69	-0.33
	-32.96	0.24		

	-33.36	1.04		
Six	-32.25	0.66	-35.5	-0.52
	-33.62	1.66	-34.34	-0.21
	-33.61	1.09	-32.61	0.04
	-33.54	1.7		
	-33.51	1.26		
Seven	-29.89	1.66	-30.72	-0.31
	-29.87	1.44	-30.87	-0.68
	-29.97	0.95	-31.01	-0.78
	-29.89	2.77		
	-30.01	2.26		
Eight	-29.23	1.66	-35.92	-0.03
	-27.93	1.4	-32.99	-0.06
	-29.08	2.41	-34.9	0.09
	-29.04	0.67		
	-29	1.48		
Nine	-31.43	1.43	-31.4	-2.68
	-31.44	0.39	-34.48	-2.54
	-31.22	0.85	-28.64	-2.78
	-31.11	1.42		
	-31.27	1.22		
Ten	-32.13	0.97	-31.9	-2.92
	-32	0.61	-32.21	-2.21
	-32.02	0.42	-32.88	-2.67
	-32.19	0.88		
	-31.43	0.9		

Eleven	-29.81	1.06	-34.01	-0.5
	-29.88	1.09	-35.4	-0.24
	-29.92	1.1	-33.16	-0.48
	-30.11	1.25		
	-29.68	1.31		
Twelve	-26.01	2.69	-34.58	-1.01
	-26.14	2.83	-32.31	-0.65
	-26.05	3.39	-32.56	-0.82
	-26.2	3.24		
	-24.16	3.52		